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Genetics and biology of mutations affecting genetic recombination, sensitivity to alkylating agents, and apurinic endonuclease in Staphylococcus aureus

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GENETICS AND BIOLOGY OF MUTATIONS AFFECTING GENETIC RECOMBINATION, SENSITIVITY TO ALKYLATING AGENTS, AND APURINIC ENDONUCLEASE IN STAPHYLOCOCCUS AUREUS

Iowa State University

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Genetics and biology of mutations affecting genetic recombination, sensitivity to alkylating agents, and apurinic endonuclease in *Staphylococcus aureus*

by

Jeffrey Ernest Tam

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INTRODUCTION

Mutants deficient in genetic recombination have been isolated and characterized in various bacteria, but detailed studies of the genetics of these mutants have been performed only in Escherichia coli and Bacillus subtilis (Clark, 1973; Venema, 1979). The recA gene of E. coli (Clark, 1971; Clark, 1973) and the recE gene of B. subtilis (Mazza et al., 1975; De Vos and Venema, 1983) are critical for genetic recombination and appear to be biochemically equivalent to each other (De Vos et al., 1983). Similar studies of genetic recombination in other bacteria have resulted in the recovery of phenotypically similar mutants, but very few of these have been as well-studied as the E. coli and B. subtilis mutants.

In Staphylococcus aureus, studies involving genetic recombination have centered on the recA1 mutation described by Wyman et al. (1974). This mutation, which is phenotypically similar to the recA and recE mutations of E. coli and B. subtilis, respectively (Wyman et al., 1974; Goering, 1979), has been useful in examining the formation of plasmid cointegrates (Novick et al., 1981), the biology of Tn554 (Phillips and Novick, 1979; Murphy et al., 1981), and recombinational repair of DNA (Thompson and Hart, 1981).

However, more extensive studies with recA1 and other rec mutations (Goering and Pattee, 1971; Inoue et al., 1972; Goering, 1979) are limited by the inability to genetically transfer these mutations. The rec mutations, such as recA1, are unselectable alleles and efficient genetic transfer of these mutations is possible only when these mutations can be cotransferred with readily selectable markers. Unfortunately, the cotransfer of recA1, or any of the other rec mutations, requires a knowledge of the chromosomal position of these mutations so that the proper selectable markers may be chosen, and to date none of these mutations are mapped. Accordingly, the goals of this study were to determine the chromosomal location of the recA1 mutation and to develop the means to readily transfer this mutation among various strains of S. aureus.

LITERATURE REVIEW

Genetic recombination in living cells has been collectively referred to as the cellular process which results in establishing new linkage relationships of genes or parts of genes (Radding, 1973). This definition of genetic recombination is applicable in events such as gene conversion (Clark, 1971), DNA repair (Hanawalt et al., 1979), and chromosomal rearrangements (Starlinger, 1977).

There are three recognized classes of genetic recombination: a) general or homologous recombination where the genophores are homologous and recombination can occur throughout their entire length b) site-specific recombination is recombination that occurs at a limited number of specific sites and c) illegitimate or nonhomologous recombination describes recombination between genophores which share little or no homology (Radding, 1973). While each of these classes of genetic recombination are important in the biology of a cell, this review will deal mainly with the genetic and molecular events of homologous recombination. Site-specific recombination, which occurs mainly among bacteriophage and viruses, has been reviewed by Miller et al. (1978) with an emphasis on bacteriophage lambda. Illegitimate recombination,

which usually describes the events in the insertion of bacteriophage Mu and transposable elements, has recently been reviewed by Kleckner (1981).

Homologous Recombination

E. coli possesses at least two pathways for homologous recombination; the RecBC pathway and the RecF pathway (Clark, 1971; Clark, 1973; Horii and Clark, 1973). The RecBC pathway, as the name implies, is dependent on the gene products of the recB and recC loci. These gene products form a complex having an ATP-dependent exonuclease activity against both double-stranded DNA (DS-DNA) and single-stranded DNA (SS-DNA). This complex, designated Exonuclease V (Exo V), also has an ATP-stimulated SS-DNA endonuclease activity and the ability to unwind superhelical DNA (Clark, 1980; Muskavitch and Linn, 1981; Muskavitch and Linn, 1982). In contrast, no gene product has yet been identified for recF, or any of the other genes making up the RecF pathway (i.e., recK and recL; Horii and Clark, 1973).

The recombination process, regardless of the pathway, consists of a number of similar events. The first event in recombination is the presynaptic phase in which the donor DNA is prepared for synapsis with its homologous region on the

chromosome. Second is synapsis, which refers to the events leading to heteroduplex formation, and includes alignment of the donor with the homologous region on the chromosome and displacement of the noncomplementary strand. The final event in homologous recombination is strand exchange between the donor and the chromosome which includes the formation of covalent bonds between the synapsed donor and the chromosome (Radding, 1982; Cox et al.,1983; Radding et al., 1983).

The best studied of the two recombinational pathways is the RecBC pathway. The RecBC pathway, which constitutes 99% of the recombination activity during E. coli conjugation (Horii and Clark, 1973), is specific for DS-DNA and results in substitution recombination (i.e., replacement of both strands of chromosomal DNA with donor DNA; Mahagan and Datta, 1979). The function of Exo V appears to be to unwind and introduce single-stranded regions into the donor DS-DNA during the presynaptic phase (Muskavitch and Linn, 1982). The RecBC pathway is not only specific for DS-DNA, but also seems to be restricted to linear DNA (Lloyd and Thomas, 1983).

Mutants in either recB or recC exhibit lethal sectoring with only 20% of the cells viable in a colony (Clark, 1973; Clark, 1980) and are sensitive to ultraviolet (UV) irradiation, x-rays, and mitomycin C (MitC) in addition to

exhibiting a reduced level of recombination (Clark, 1971). When revertants of a recB recC mutant were isolated, the revertants were found to be pseudorevertants with a mutation in the sbcB locus which was allowing the expression of a second recombination pathway (Horii and Clark, 1973). This second pathway was the RecF pathway.

The RecF pathway is genetically and mechanistically distinct from the RecBC pathway except for the requirement that each pathway has for the recA protein. The RecF pathway requires at least two loci in additional to recF and the sbcB mutation, recK and recL (Horii and Clark, 1973). The RecF pathway incorporates only SS-DNA into the chromosome, and it is believed that the sbcB mutation is needed either to prevent degradation of the donor SS-DNA (Mahagan and Datta, 1979) or to derepress the RecF pathway (Lloyd and Thomas, 1983). Since none of the components of the RecF pathway have been isolated, the molecular events of this pathway remain unknown. It is believed, however, that the components of this pathway are required in the presynaptic phase of recombination (Mahagan and Datta, 1979).

Whereas both of these pathways have a requirement for the recA protein, the RecF pathway requires stoichiometrically greater amounts of this protein with the entire recombination

process proceeding at a much slower rate, and unlike the RecBC pathway, the RecF pathway appears to be inducible (Mahagan and Datta, 1979; Lloyd and Thomas, 1983). Evidence for induction of the RecF pathway comes from work with the mutation rec-259, the gene affected by rec-259 appears to be under the control of the lexA protein and is transcribed under conditions similar to those which induce the SOS response (Lloyd et al., 1983). Phenotypically, the rec-259 mutation imparts sensitivities to UV irradiation and MitC which are greater than those observed in a recB$^-$ recC$^-$ sbcB$^-$ mutant. Furthermore, cells which carry rec-259 in a recB$^+$, recC$^+$, and sbcB$^+$ background remain sensitive to UV irradiation even though they are Rec$^+$ (Lloyd et al., 1983). Based on the inducible nature of the RecF pathway and the sensitivity of the rec-259 mutants to UV irradiation, it is likely that the RecF pathway is the main pathway for recombination repair or post-replicative repair in E. coli while the RecBC pathway is mainly utilized for recombination during conjugation (Lloyd et al., 1983; Lovett and Clark, 1983).

recA

In E. coli, the recA locus has long been known to be required for recombination and DNA repair. By the late 1960s,

mutations in recA had been isolated, mapped, and their phenotypes well cataloged (Clark, 1971). However, the identity of the recA gene product was unknown until McEntee (1977) demonstrated that protein-X, which was induced during the SOS response (Witkin, 1976), was encoded by recA. The participation of the recA gene product in recombination was first described by Kobayashi and Ikeda (1978). These investigators, using a temperature-sensitive recA mutant, demonstrated that recombination occurred at permissive temperatures even when transcription and translation were inhibited. However, at restrictive temperatures there was no recombination with or without inhibition of transcription and translation. The hypothesis formed by Kobayashi and Ikeda (1978) was that the recA protein of E. coli was not a only a regulator of recombination, but also an essential component of recombination and normally present at a cellular concentration that made induction of the recA gene unnecessary for recombination to occur.

The hypothesis that the recA protein had an integral role in recombination was supported by Shibata et al. (1979). These investigators demonstrated that purified recA protein and ATP were sufficient to mediate homologous pairing between SS-DNA and superhelical DS-DNA in vitro. These experiments

also demonstrated that the role of the recA protein was in the synapse phase of recombination.

With the isolation of the recA protein, research on this gene diverged into three main areas: a) physiochemical aspects of the recA protein, b) molecular role of recA in recombination, and c) the regulation of the SOS and other DNA repair processes by recA. The physiochemical characteristics of the recA protein have been reviewed by McEntee and Weinstock (1981). Briefly, the recA protein is a 37,842 dalton protein with the ability to bind SS-DNA, and NTP (nucleotide triphosphates). In the presence of SS-DNA and ATP, the recA protein will bind DS-DNA homologous to the SS-DNA and will mediate strand assimilation or exchange (McEntee and Weinstock, 1981; Radding, 1982). In addition, the recA protein has a protease activity which is specific for the lexA gene product and the repressor proteins of the temperate bacteriophages lambda and P22 (Little et al., 1981; McEntee and Weinstock, 1981); the lexA gene product is the repressor of the SOS response (Little and Mount, 1982; Little, 1983; Little et al., 1981; McEntee and Weinstock, 1981). The nucleotide sequence and the amino acid sequence of the amino and carboxyl termini have been determined (McEntee and Weinstock, 1981) and recently, the various functions of the

recA protein were assigned to domains on the recA protein (Kawashima et al., 1984).

The role of the recA protein in DNA repair has received a great deal of attention since Witkin (1976) reviewed the SOS response. Generally, the role of recA in DNA repair appears to be at both the enzymatic level and at the regulatory level (Hanawalt et al., 1979). The regulatory function of the recA protein is associated with the proteolytic activity of this protein towards the lexA gene product. The lexA repressor protein negatively controls the transcription of at least 11 genes (Little and Mount, 1982) including recA and lexA (i.e., autoregulation). While recA is regulated by the lexA repressor, the celluar concentrations of recA protein in uninduced cells is sufficient to carry-out homologous recombination (Kobayashi and Ikeda, 1978; Walker, 1984) without the need for further induction of the recA gene. Upon DNA damage or inhibition of DNA replication, the protease activity of the endogenous recA protein is induced and cleavage of the lexA repressor protein occurs. This cleavage of lexA repressor protein drastically lowers its cellular concentration and results in induction of the genes controlled by this protein (Little and Mount, 1982; Walker, 1984). The induction of the genes under lexA control is commonly known as

the SOS response and results in: a) enhancement of both excision and postreplication repair, b) increased mutagenesis, c) inhibition of cell division, d) prophage induction, e) cessation of respiration, f) Weigle reactivation (W-reactivation), and g) induction of stable DNA replication (Witkin, 1976; Little and Mount, 1982; Walker, 1984).

Activation of the recA protease is a complex series of interactions between polynucleotides and other proteins. Oishi et al. (1981) showed that recA protease activity could be induced by: 1) strand scission of the DNA, 2) chemically induced DNA damage, or 3) inhibition of replication. They also observed that induction of the recA protease was dependent on the RecBC exonuclease, Exo V. The current hypothesis is that single-stranded oligomers released during degradation of the damaged DNA by Exo V bind to the recA protein and activate the recA protease (Oishi et al., 1981). This hypothesis was supported by the ability to induce the SOS response in permeabilized E. coli cells by the addition of synthetic oligomers of 6 to 18 bases in length (Oishi et al., 1981). In addition to RecBC-dependent induction, McPartland et al. (1980) showed that the recA protease was also activated by a RecF-dependent mechanism. Generally, RecBC-dependent induction of the recA protease seems to be favored when DNA

replication is inhibited, while the RecF-dependent induction is favored after UV irradiation (McPartland et al., 1980).

The proteolytic activity of the recA protein is controlled by single-strand-binding protein (ssb protein; Cohen et al., 1983), the concentration of single-stranded oligomers or polynucleotides, and an unidentified product of the SOS response (Little, 1983). Cohen et al. (1983) demonstrated that the ssb protein controls the induction of the recA protease by affecting the extent to which the recA protein can bind SS-DNA and its conformation when bound. When the concentration of ssb protein is high, the recA protease is induced. However, when ssb protein concentrations are low, the induction of the recA protease, even in the presence of single stranded oligomers and polynucleotides, will not occur (Cohen, et al., 1983). Mutants of recA demonstrate the loss of SOS functions and are much more sensitive to a wide variety of DNA damage, in addition to being unable to induce prophage, and showing an increase in degradation of their DNA after UV irradiation (reckless DNA repair; Clark, 1971; Clark, 1973; Eisenstark, 1977).

In addition to the regulation of DNA repair, the recA protein is critical for recombinational repair. This repair process is believed to be mechanistically the same homologous

recombination, but the fidelity of recombination repair appears to be low, resulting in a higher incidence of mutations (Hanawalt et al., 1979; Livneh and Lehman, 1982; Lloyd et al., 1983).

The molecular role of the recA protein in recombination has been determined mainly from in vitro studies and recently, two models of homologous recombination have evolved. In both models, the recA protein plays an integral role and in the following discussion the two models will be presented with an emphasis on the recA protein.

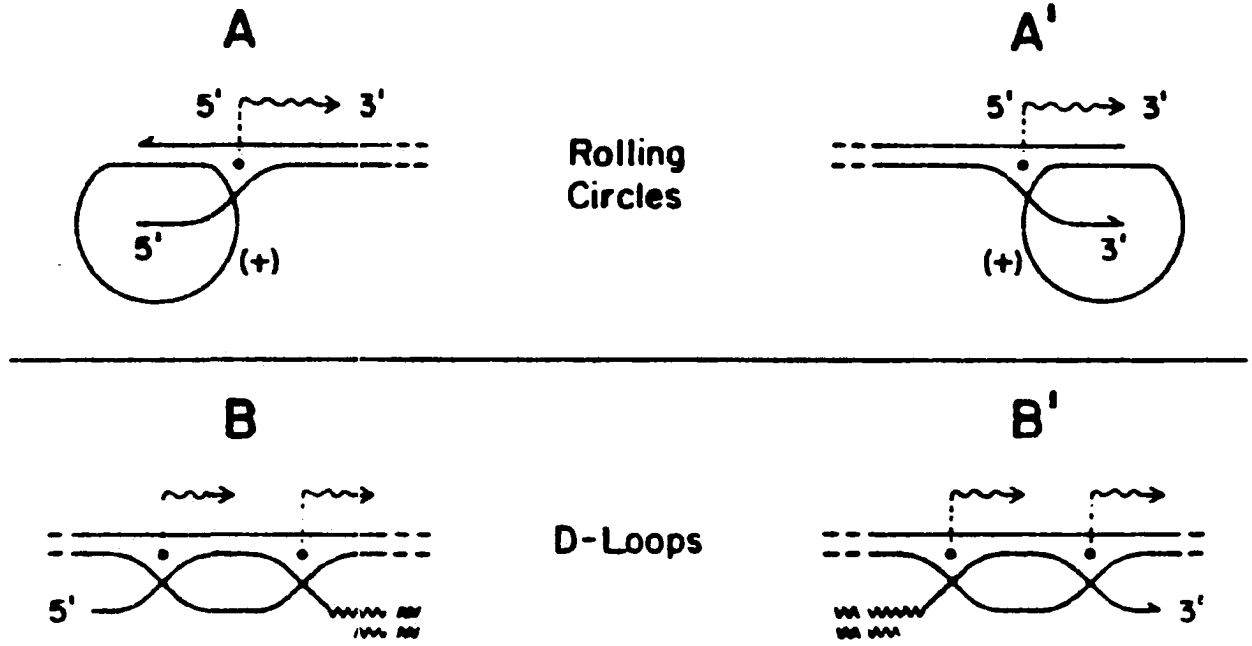
The first model was postulated by Radding et al. (1983). As previously stated, the recombination process can be divided into three phases. During the first phase, presynapsis, the recA protein polymerizes onto the SS-DNA donor (i.e., RecF pathway) or single-stranded regions of a DS-DNA donor (i.e., RecBC pathway), forming a "filament" (Radding et al., 1983). The filament also binds ATP, which is required for the subsequent phases (Radding, 1982; Cox et al., 1983), and ssb protein, which stabilizes the filament and increases the efficiency of the subsequent reactions (Cox et al., 1983)

The next two phases, synapsis and strand exchange, require the hydrolysis of ATP and result in the formation of a heteroduplex between the donor and the chromosome (Radding,

1982; Radding et al., 1983). In the synapsis phase, the (ATP)-(recA)-(SS-DNA donor)-(ssb protein) complex formed during the presynaptic phase (presynaptic complex) binds at the site of chromosomal homology. Binding or synapsis occurs at the 3'-end of the chromosomal site of homology through Watson-Crick base pairing between the complementary strand of the chromosome and the donor with displacement of the noncomplementary strand of the chromosome (Wu et al., 1982). In this model, detection and alignment of the donor with its homologous region on the chromosomal occurs by a processive mechanism requiring the recA protein and the formation of a structural intermediate between the presynaptic complex and the chromosome. It is this intermediate structure which "searches" the chromosome for a region homologous to the donor (Gonda and Radding, 1983). The processive mechanism is in contrast to a series of random association-dissociation events between the presynaptic complex and the chromosome. Strand exchange is polar and progresses 5' to 3' relative to the synapsis site (Fig. 1; Cox et al., 1983), requires the hydrolysis of ATP, and the unwinding activity of the recA protein (Ohtani et al., 1982; Radding, 1982). The strand-exchange process is stimulated by ssb protein which also reduces the amount of ATP consumed (Cox et al., 1983).

Figure 1. Polar strand exchange and D-loop formation as hypothesized by the Radding model of homologous recombination

Strand exchange is shown with SS-DNA as a circular form (1A), and as DS-DNA (1B); the complementary strand of the donor was omitted for clarity. Zig-zag lines associated with the donor DS-DNA (1B) represents heterologous DNA. Wavey arrow shows 5' to 3' direction of strand exchange, and (●) represents the points of synapsis. The figures 1A' and 1B' are examples of strand exchange when synapsis occurs at or near the 3' terminus of the recipient molecule. Figure 1B and 1B' show the formation of the hypothetical D-loop by displacement of the noncomplementary strand of the recipient by donor DNA (taken from Wu et al., 1982).

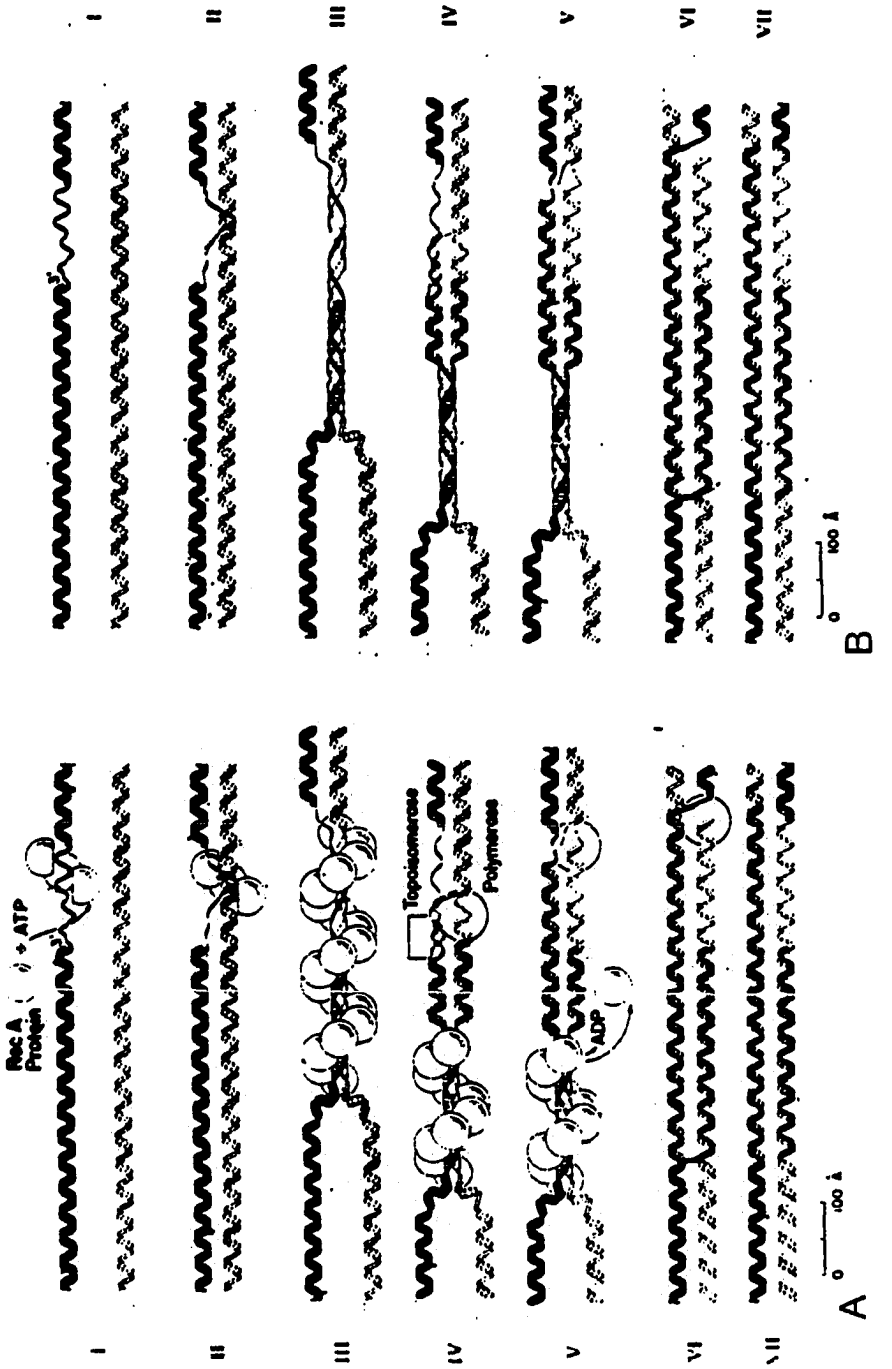

A
5'
3'
5'
(+)
Rolling
Circles
A'
5'
3'
(+)
3'
B
5'
D-Loops
B'
3'

The final step in the recombination process is ligation of the donor into the chromosome.

The second model for homologous recombination has been postulated by Howard-Flanders et al. (1984). This model is based on much of the same data as the Radding model, but also incorporates data from electron microscopic and X-ray crystallographic studies (Howard-Flanders et al., 1984). The major differences between the Howard-Flanders model and the Radding model are in the synapse and strand exchange events. In the Howard-Flanders model, the alignment of the presynaptic complex to its homologous site on the chromosome is thought to occur by random association-dissociation and not by the formation of an intermediate structure described in the Radding model (Howard-Flanders et al., 1984). Synapsis then occurs between the presynaptic complex and the chromosome in the major groove of the double helix (Fig. 2) rather than by the Watson-Crick pairing and displacement of the non-complementary strand described in the Radding model. As the presynaptic complex winds itself around the double helix of the chromosome, following the major groove, strand exchange occurs and the noncomplementary strand is displaced (Fig. 2). Each monomer of the recA protein is believed to have two DNA binding sites: one site binds to the single-stranded donor or

Figure 2. Schematic of the Howard-Flanders model of homologous recombination between two DS-molecules

A. Model complete with proteins. B. Model with proteins omitted for clarity. I-II. Synapsis of the exogenote (heavy line) with the recipient (light line) requires ATP, a SS-DNA region on the exogenote, and recA protein polymerized onto the SS-DNA. Binding of the exogenote is in the major groove at the site of homology, the (SS-DNA)-(recA protein) filament is shown following the major groove of the recipient double helix. Only a few recA protein monomers are shown; actually a protein covered filament would be observed. III-VI. Strand exchange mediated by recA protein as hypothesized in Fig. 2. Topoisomerase is required to relax the unraveling exogenote and DNA polymerase is needed to fill in the SS-DNA gaps. VII. Enzymatic separation of the heteroduplex (taken from Howard-Flanders et al., 1984).


I
II
III
IV
V
VI
VII
Rec A Protein
ATP
Topoisomerase
Polymerase
ADP
A
0
100 Å
B
0
100 Å

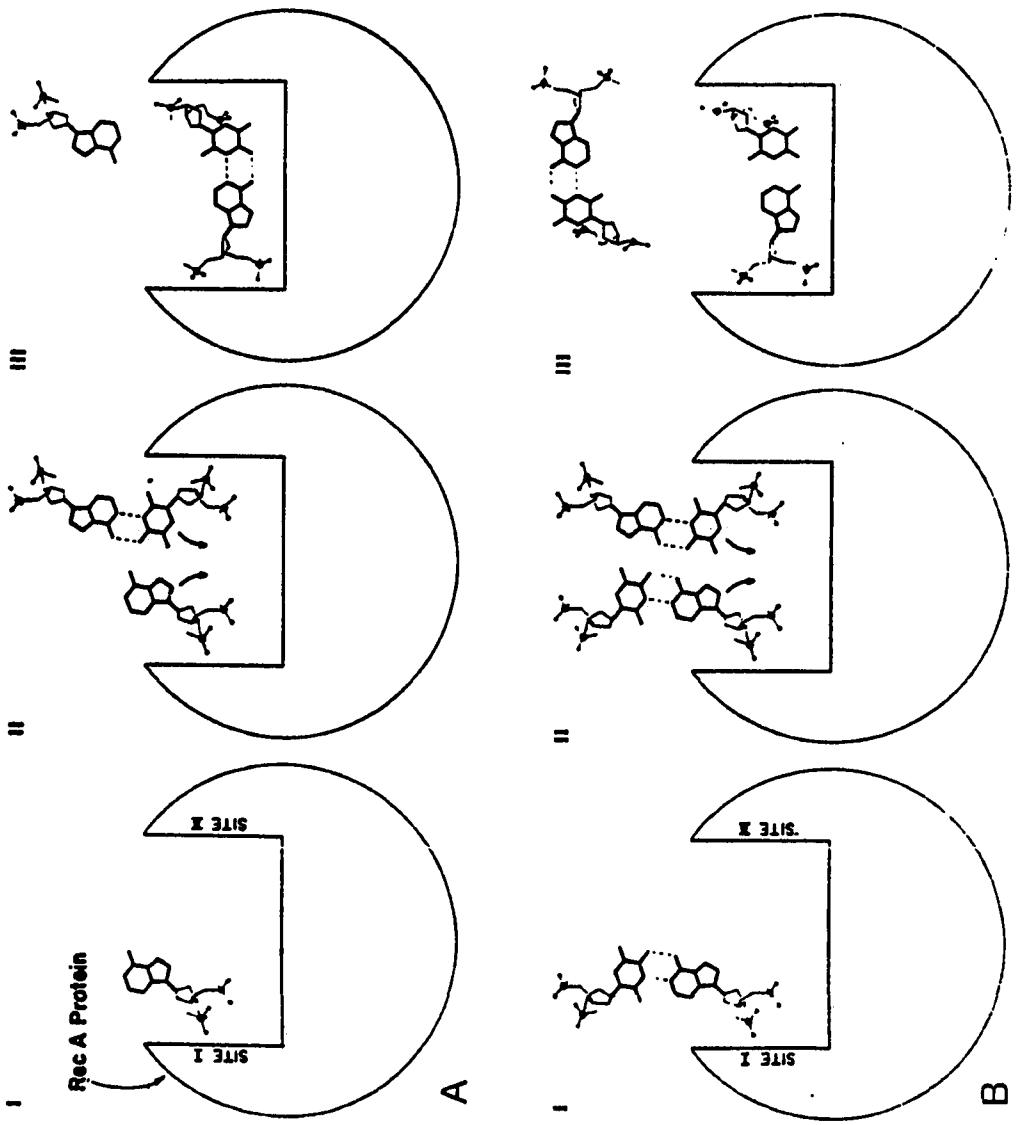
the presynaptic complex, the other site binds the strand of the chromosome complementary to the donor, and by bringing these strands into close proximity to each other the recA protein forces pairing to occur between the donor DNA and the chromosome (Fig. 3). This event requires the hydrolysis of ATP, but does not require the unwinding activity by the recA protein (Howard-Flanders et al., 1984). To determine which model, if either, is correct will require elucidating how strand exchange occurs at the molecular level, the mechanisms of DNA binding and release by the recA protein, and the role of ATP in these events (Howard-Flanders et al. 1984).

recA-Like Genes in Other Bacteria

Among gram negative bacteria, recA-like genes have been identified in Agrobacterium tumefaciens (Klapwijk et al., 1979), Haemophilus influenzae (Koositra et al., 1983), Proteus mirabilis (Eitner et al., 1982), and Salmonella typhimurium (McPhee, 1970). Similar genes have also been identified in the gram positive bacteria Bacillus subtilis (Mazza et al., 1975; De Vos and Venema, 1983), Deinococcus radiodurans (Moseley and Copland, 1975), Staphylococcus aureus (Inoue et al, 1972; Wyman et al., 1974; Goering, 1979), Streptococcus faecalis (Yagi and Clewell, 1980), and Streptococcus sanguis

Figure 3. Diagrammatic representation of the hypothesized DNA binding and exchange mechanism of *E. coli recA* protein

A. Binding of SS-DNA exogenote to site I occurs during presynaptic phase (I). During synapsis the complementary strand of the chromosome is bound (II), and forced to form Watson-Crick pairing with the exogenote (III). B. Events shown are analogous to those in A, but occur between a DS-exogenote and DS-chromosome as in reciprocal recombination (taken from Howard-Flanders et al., 1984).


I
II
III
Rec A Protein
SITE I
SITE II
A
B

(Raina and Macrina, 1982). Rec⁻ mutants have been isolated in all of these bacteria which demonstrated at least a 100-fold decrease in recombination activity, and increased sensitivity to UV irradiation or methyl methanesulfonate (MMS). However, only in H. influenzae (Kooistra and Venema, 1974), P. mirabilis (Eitner et al., 1982), B. subtilis (Sadaie and Kada, 1976; De Vos and Venema, 1983), and S. aureus (Inoue et al., 1972; Wyman et al., 1974; Goering, 1979) do the mutants exhibit UV-induced DNA degradation and the inability to induce prophage.

The molecular events of recombination in S. sanguis, H. influenzae, and B. subtilis have been characterized and they appear to be analogous to those of E. coli. The phases of recombination in these bacteria are very similar to those already described for E. coli with definite presynaptic (eclipse), synaptic, and strand-exchange phases (Venema, 1979). The major difference between E. coli and these bacteria is that in B. subtilis and S. sanguis the DNA participating in the eclipse phase is SS-DNA (Popowski and Venema, 1978; Raina and Ravin, 1978), while H. influenzae, like E. coli, uses DS-DNA (Venema, 1979; Noteborn et al., 1981). Although the level of knowledge of the molecular events of homologous recombination in these bacteria is still

rudimentary when compared to E. coli, these Rec⁻ mutants appear to be analogous to the recA mutants of E. coli in that the ability of these mutants to mediate synapsis and/or strand exchange is impaired (Kooistra and Venema, 1974; Venema, 1979; Raina and Macrina, 1982; De Vos and Venema, 1983).

Recently, the recA⁺ gene from E. coli has been cloned into P. mirabilis (recA⁻) and B. subtilis (recE⁻). Eitner et al. (1982) showed that the cloned recA⁺ gene of E. coli was expressed and complemented the recA mutation in P. mirabilis. In a similar experiment, E. coli recA⁺ was fused to the spo2 promotor of B. subtilis and was shown to restore the recombination and DNA repair activity of B. subtilis recE mutants (De Vos et al., 1983).

In both of these reports, it is apparent that the functions and active sites of the recA protein of E. coli and the recA-like proteins of P. mirabilis and B. subtilis are complementary. The ability of E. coli recA protein to complement P. mirabilis is not unexpected since the recA protein of P. mirabilis is physiochemically identical to the recA protein of E. coli (Eitner et al., 1982; West and Little, 1984). However, the B. subtilis recE protein and E. coli recA protein are vastly different physiochemically (De Vos and Venema, 1983; De Vos et al., 1983) and yet, the E. coli recA⁺

gene can still complement B. subtilis recE mutants. These results suggested that the active sites of these recA-like proteins were probably conserved even if the overall physical structure was not (De Vos et al., 1983).

Recombination Deficient Mutants of Staphlyococcus aureus

A number of Rec⁻ strains have been isolated in S. aureus, and in each instance the isolation was based on sensitivity to either UV irradiation (Goering and Pattee, 1971; Inoue et al., 1972; Wyman et al., 1974) or N-methyl-N-nitro-N-nitroso-guanidine (MNNG; Goering, 1979). The first mutation reported to cause a deficiency in recombination in S. aureus was uvs-1 described by Goering and Pattee (1971) in strain 112. While the uvs-1 mutant was deficient in recombination, it more closely resembled recB or recC mutants in E. coli than recA mutants (Wyman et al., 1974). Mutants which exhibit phenotypes similar to recA mutations in E. coli have been isolated by Inoue et al. (1972) and Wyman et al. (1974); of these, the recA1 mutant described by Wyman et al. (1974) has been the best characterized. The recA1 mutant was not only sensitive to UV irradiation and Rec⁻, but was also unable to induce prophage, demonstrated lethal sectoring, was sensitive to MitC, and impaired in DNA modification (Wyman et al., 1974). Goering (1979) later showed that this mutant was

sensitive to MNNG and underwent reckless DNA repair when UV irradiated. All of these phenotypes, except for the loss of DNA modification, were very similar to those of E. coli recA mutants (Clark, 1971; Clark, 1973; Eisenstark, 1977).

Recently, S. aureus was examined for the presence of inducible DNA repair functions similar to the SOS response in E. coli. Thompson and Hart (1981) examined S. aureus for the presence of inducible UV mutagenesis and W-reactivation, functions which in E. coli require the presence of recA protein. These investigators found that both of these events occurred in wild-type S. aureus, but were absent in recA1 strains. However, in S. aureus, these events were constitutive and not inducible as was expected (Thompson and Hart, 1981). The presence of these two events supported the claim by Wyman et al. (1974) that S. aureus had a recA-like gene and that recA1 was a mutation in this gene, but the constitutive nature of these events indicated that there were some very basic differences in recombination, DNA repair pathways, and the functions of the recA-like protein in S. aureus when compared to E. coli (Thompson and Hart, 1981). Aside from this report, very little is known about the genetics or molecular biology of homologous recombination in S. aureus.

Repair of Apurinic and Apyrimidinic Sites in DNA

The depurination and depyrimidination of DNA can occur in both prokaryotic and eukaryotic cells. The sites formed by these events are commomly referred to as apurinic and apyrimidinic sites (AP sites), respectively (Lindahl, 1979). An AP site consists of a site in the DNA molecule which lacks the base (e.g., adenine, thymine, etc.) and is formed either spontaneously or enzymatically. Spontaneous formation of AP sites occurs under most conditions, but the rate of formation is dependent on the temperature at which the cell is growing. For example, at 37°C E. coli will generate one AP site per generation, while Bacillus stearothermophilus growing at 70°C will form 50 AP sites per generation, and Thermus thermophilus growing at 85°C will generate 300 AP sites per generation (Lindahl, 1979).

Enzymatic formation of AP sites is usually caused by DNA-N-glycosylase activity. These enzymes recognize and remove aberrant bases from the DNA molecule through cleavage of the glycosyl bond, but leave the phosphate backbone intact (Lindahl, 1979). The best characterized DNA-N-glycosylases are those which recognize the spontaneously formed deoxyuracil, deoxyhypoxanthine, or deoxyxanthine in the DNA molecule (Lindahl, 1979; Lindahl, 1982). Other DNA-N-

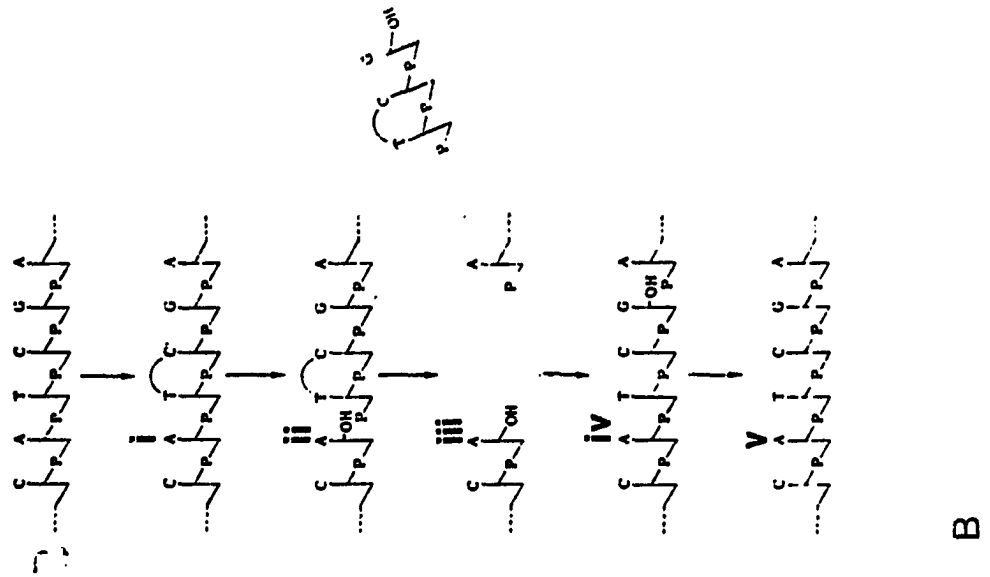
glycosylases have been identified which recognize bases in the DNA molecule altered by chemical treatments or UV irradiation. Examples of such enzymes are 3-methyl-adenine-DNA-glycosylase active on alkylated DNA (Lindahl, 1979) and the thymine-dimer recognizing DNA-glycosylases of either Micrococcus luteus (Laval and Pierre, 1978) or the E. coli bacteriophage T4 (McMillan et al., 1981; Warner et al., 1981). The formation of AP sites by these enzymes is believed to be the first step in the base excision repair process (Friedberg et al., 1978) which should not be confused with nucleotide excision repair (Fig. 4).

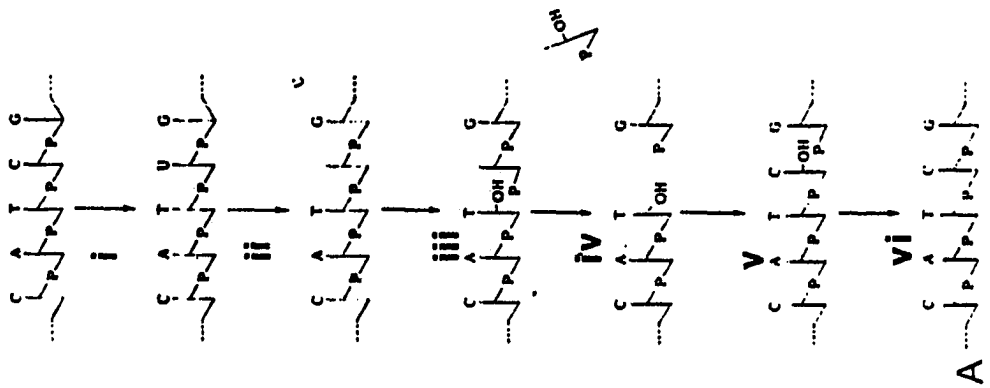
The introduction of AP sites into DNA, whether by spontaneous or enzymatic means, are potentially lethal or mutagenic (Lindahl, 1979; Sagher and Strauss, 1983; Schaaper et al., 1983). However, when the rate of spontaneous AP site formation was compared to the spontaneous mutation rate there was no relationship between the two (Lindahl, 1979). It was presumed that the lack of mutagenesis associated with the formation of these lesions was a consequence of a highly efficient and specific repair mechanism (Schaaper et al., 1983).

A general scheme of AP site repair has been developed in E. coli and relies on the presence of a class of enzymes

Figure 4. Excision repair pathways

A. Base excision repair. Spontaneous deamination of cytosine results in the formation of deoxyuracil (i) which is removed by a uracil-DNA-glycosylase(ii). The resulting AP site is then recognized by an AP endonuclease and a nick introduced on the 5' side of the site (iii). Exonuclease acitivity then removes the deoxyribose- phosphate moiety (iv) and the gap is repaired by DNA polymerase (v) and DNA ligase (vi). B. Nucleotide excision repair. A pyrimidine-dimer is formed by UV irradiation (i) and and a nick is introduced on the 5' side of the dimer by an UV endonuclease (ii). A gap formed by an exonucleolytic removal of the dimers and adjacent nucleotides (iii), with repair of the DNA by DNA polymerase (iv) and DNA ligase (v) similar to base excision repair (taken from Lindahl, 1979).


i
ii
iii
iv
v
B


i
ii
iii
iv
v
vi
A

having AP endonucleolytic activity (Lindahl, 1982). In E. coli three endonucleases having AP endonucleolytic activity (AP endonuclease) have been identified and are: 1) Exonuclease III (Exo III) which has also been termed Endonuclease II and Endonuclease VI (Yajko and Weiss, 1975; Gossard and Verly, 1978; Ljundquist et al., 1976), 2) Endonuclease III (Ljundquist et al., 1976; Lindahl, 1982), and 3) Endonuclease IV (Ljundquist, 1977).

Of the three AP endonucleases, Exo III is the best characterized. It is encoded by the xthA locus (Yajko and Weiss, 1975; Ljundquist et al., 1976) and has four known enzymatic activites: 3' to 5' exonuclease, AP endonuclease, RNase H, and DNA-3-phosphatase (Lindahl, 1982). Exo III removes AP sites from DNA by introducing a nick in the phosphate backbone on the 5' side of the AP site (Fig. 4A), which is followed by formation of a gap by removal of the nucleotides on the 3' side of the nick (Lindahl, 1979). Presently, it is unknown whether the formation of the gap is by the 3' to 5' exonuclease of Exo III or by a second exonuclease (Taylor and Weiss, 1982). It is worth noting that Exo III is the only AP endonuclease with an exonucleolytic activity which may be vestigial (Taylor and Weiss, 1982). In the latter stages of AP site repair, the enzymes for filling

in the gap and ligation appear to be the same as those used in the nucleotide excision pathway, DNA polymerase I and DNA ligase (Lindahl, 1979; Warner et al., 1980; Friedberg et al., 1981). Repair of AP sites by the other AP endonucleases is similar to the process just described, but the other enzymes differ in their positioning of the nick relative to the AP site (Warner et al., 1980).

Exo III is the major AP endonuclease in E. coli (Lindahl et. al, 1976; Gossard and Verly, 1978; Lindahl, 1982) and mutants can be isolated by their sensitivity to MMS (Ljundquist et al., 1976; Gossard and Verly, 1978). In addition, Exo III mutants are sensitive to treatment with nitrous acid (Da Roza et al., 1977), hydrogen peroxide (Demple et al., 1983), and near-UV irradiation (Sammutano and Tuveson, 1983). However, these mutants do not show any decrease in cell viability under normal culture conditions (Yajko and Weiss, 1975; Ljundquist et al., 1976), suggesting that Exo III is expendable with a deficiency in Exo III compensated for by the two minor AP endonucleases (Ljundquist et al., 1976; Lindahl, 1979).

Since both prokaryotes and eukaryotes appear to form AP sites it is not surprising that in all cells examined, AP endonucleases have been present. For example, AP

endonucleases have been identified in cell-free extracts of mammalian and plant tissue as well as in H. influenzae, B. stearothermophilus, and M. luteus (Lindahl, 1979). However, the genetics of the enzymes in these organisms are virtually unknown.

Chromosomal Mapping in Staphylococcus aureus

Chromosomal mapping in the Staphylococcus aureus began with the introduction of generalized transduction (Morse, 1959). Through the use of generalized transduction it has been possible to elucidate the genetics of tryptophan biosynthesis (Ritz and Baldwin, 1962; Proctor and Kloos, 1970) and isoleucine-valine biosynthesis (Smith and Pattee, 1967; Pattee et al., 1974), to map the histidine operon (Kloos and Pattee, 1965), and to determine the organization of the thy-trp region (Schroeder and Pattee, 1984). However, in spite of its extensive use in the development of S. aureus genetics, generalized transduction in S. aureus suffers from two major drawbacks. The first is the small amount of DNA that can be packaged into the transducing bacteriophage. Lacey (1975) reported that transducing bacteriophage of S. aureus were capable of packaging a fragment of only 30 Mdal which greatly reduces the size of the linkages which can be

examined. Secondly, Pattee et al. (1968) have presented evidence based on mapping studies of the histidine operon of S. aureus, that the transducing bacteriophage were made up of a homogeneous population of DNA fragments rather than a random population. It has been suggested that favored sites exist on the S. aureus chromosome from which packaging of chromosomal DNA into the transducing bacteriophage begin (P. A. Pattee, personal communication, Iowa State University, Ames, Iowa). A similar situation exists in the generalized transducing bacteriophage P22 of Salmonella typhimurium. In P22, pac sites are present on the chromosome of S. typhimurium which appear to be the favored chromosomal sites for the initiation of chromosomal DNA packaging into P22. The presence of these sites strongly bias the data obtained from transductions using P22 (Schmeiger, 1982).

Genetic transformation of S. aureus NCTC 8325 was introduced by Lindberg et al. (1972). Transformation of this strain of S. aureus was dependent on the recipient cells being lysogenic for either ϕ11 or 83A (Sjostrom et al., 1973; Sjostrom and Philipson, 1974). Later it was shown that competence could be induced in this strain through the addition of Ca^{2+} and a serological group B bacteriophage (Thompson and Pattee, 1977). It now appears that competence

is induced not by the whole bacteriophage, but by the bacteriophage attachment organelle (Thompson and Pattee, 1981; Birmingham and Pattee, 1981; Allen, 1982). However, the mechanisms of competence induction and DNA uptake are still unknown (Pattee, personal communication, Iowa State University, Ames, Iowa).

The use of genetic transformation for genetic mapping was initiated by Pattee and Neveln (1975) who established the existence of three independent linkage groups in S. aureus (Fig. 5). As the number of genetic markers associated with these linkage groups increased (Pattee, 1976; Kuhl et al., 1978) they were subdivided into segments flanked by markers which were readily selectable and cotransformable (Pattee and Glatz, 1980). With selection for the flanking markers followed by screening for the desired phenotype, it was possible to map the genes for the production of alpha-toxin (hla; Brown and Pattee, 1980) and entertoxin A (entA; Pattee and Glatz, 1980; Mallonee et al. 1982).

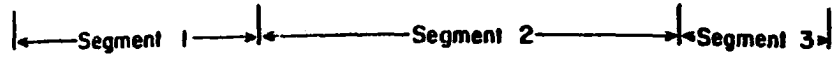
The introduction of mutagenesis using the transposable element Tn551 (encodes for macrolide, lincosamide, and streptogramin resistance) significantly increases the number of chromosomal markers within the three linkage groups (Pattee, 1981). However, the increase in the number of

Figure 5. Schematic of the three independent linkage groups described by Pattee and Neveln (1975)

Boldface loci are the selectable or scorable markers which delineate a segment. Segments are identified by the numbers shown under each linkage group in small print (taken form Pattee and Glatz, 1980).

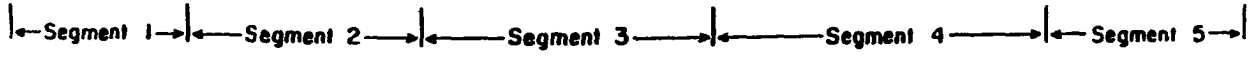
LINKAGE GROUP:

I Ω11 *thy-101* (*thrA* / *lys*) Ω8 *trp* *thrB106* Ω40



II *uraA141* *NsG15* *nov* *pur* *mec-4916*

III Ω34 *ala* *rib-127* Ω5 *tmn-3106* *attφ11* Ω1 *purB110* *bla* *hla* *ilv-129* *pig-131*



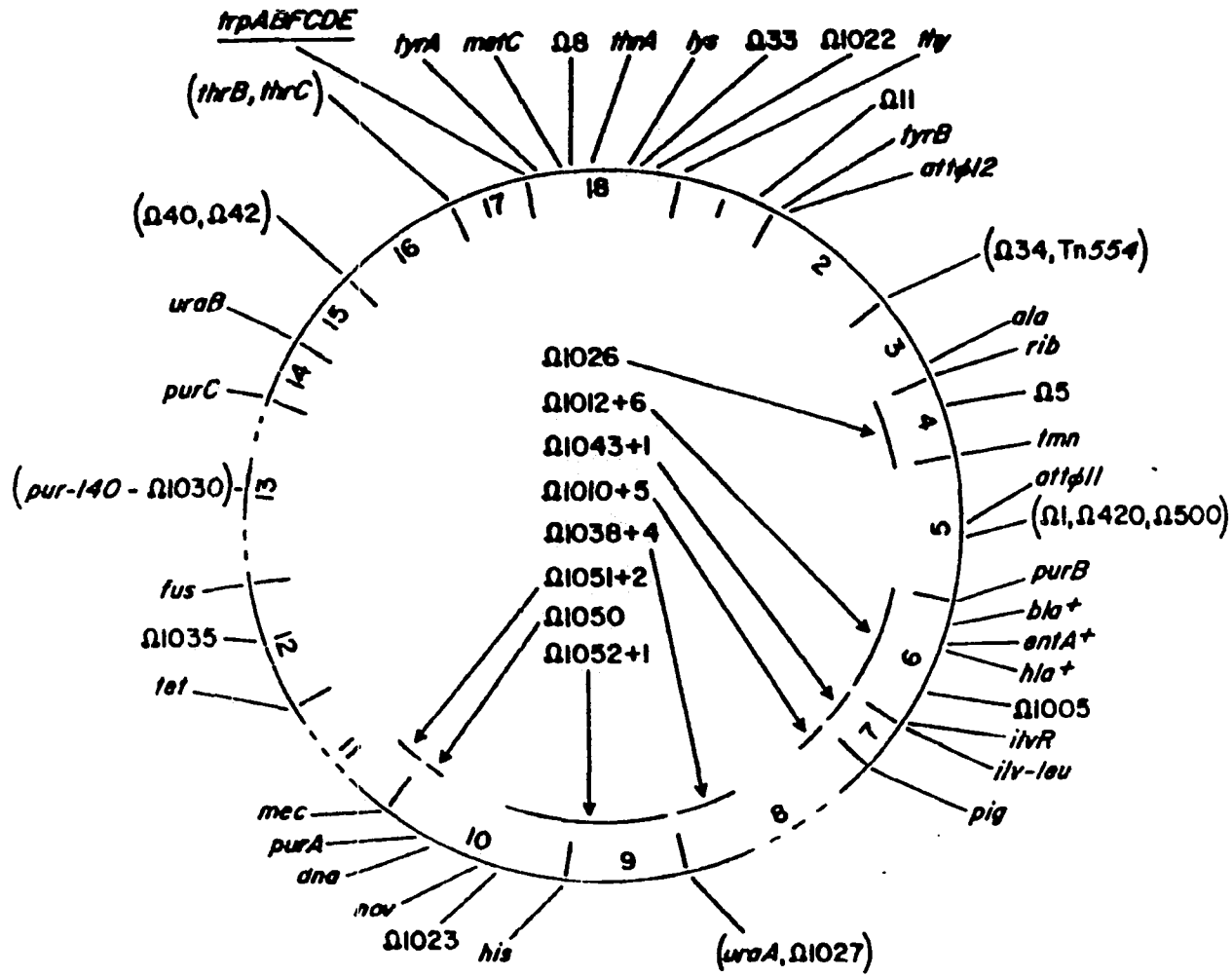
markers also increased the complexity of these linkage groups and made mapping of new markers by transformation more laborious (Stahl and Pattee, 1983a).

Recently, two new approaches have been applied to S. aureus in an effort to make mapping more efficient. The first was the development of protoplast fusion analysis which was used to predict the linkage of previously unlinked or unmapped markers to or within one of the three known linkage groups (Stahl and Pattee, 1983a). Using protoplast fusion analysis concomitant with the isolation of high molecular weight transforming DNA, Stahl and Pattee (1983b) were able to show linkage between tyrB282::Tn551 ermB321 and Ω[chr::Tn551]34. This allowed the formation of a single linkage group from linkage groups I and III, thus reducing the number of linkage groups in S. aureus from three to two (Fig. 6).

The second approach to mapping was the development of a protocol to isolate insertions of Tn551 which were cotransformable with unselectable determinants (Luchansky and Pattee, 1984). For example, though the isolation of DNA from a population of cells containing a collection of random insertions of Tn551, it was possible to isolate an insertion, Ω[chr::Tn551]1035, which cotransformed with fus-149, an unselectable allele encoding for fusidic acid resistance.

Figure 6. Schematic of the chromosome of S. aureus NCTC 8325

Numbers around the inner perimeter represent the segment numbers of the various linkage groups (see Fig. 5). Linkage groups I and III were linked into a contiguous region by computer-assisted protoplast fusion analysis and by transformation (Stahl and Pattee, 1983a; Stahl and Pattee, 1983b), shown by the solid line extending from purC to Ω1010. Broken lines indicate regions where linkage has not been established (segments 8, 11, and 13), and the orientation of markers within parentheses relative to the adjacent markers have not been determined. Sites of Tn551 insertion are designated by Ω followed by an isolation number (except Ω420 and Ω500 which are insertions of the plasmid pI258). The Tn551 insertions Ω1010 through Ω1053 (shown inside the circle) are insertions isolated on the basis of their proximity to markers of interest (taken from Luchansky and Pattee, 1984).


trpABFCDE
tyrA
metC
Ω8
thrA
lys
Ω33
Ω1022
thy
(thrB, thrC)
Ω11
tyrB
attφ12
(Ω40, Ω42)
(Ω34, Tn554)
uraB
ala
rib
purC
Ω1026
Ω1012+6
Ω1043+1
Ω1010+5
Ω1038+4
Ω1051+2
Ω1050
Ω1052+1
Ω5
tmn
attφ11
(Ω1, Ω420, Ω500)
(pur-140 - Ω1030)
purB
bla+
entA+
hla+
fus
Ω1035
tet
Ω1005
ilvR
ilv-leu
pig
mec
purA
dna
nov
Ω1023
his
(uraA, Ω1027)
18
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17

This insert near fus-149 made it possible to efficiently transfer fus-149 by selecting for Ω[ohr::Tn551]1035 and scoring for Fus^r (Luchansky and Pattee, 1984).

MATERIALS and METHODS

Bacterial Strains

The strains of Staphylococcus aureus and Bacillus subtilis used in this study, with their genotypes and origins, are listed in Table 1. The nomenclature used was as recommended by Demerec et al. (1966), Campbell et al. (1979), and Novick et al. (1979). Many of these strains have chromosomal insertions of Tn551, a transposon which carries the ermB gene encoding for erythromycin resistance (Em^r; Novick, 1967; Novick, 1974; Pattee et al., 1977; Novick et al., 1979; Pattee, 1981). Three classes of insertions were used in this study: 1) silent chromosomal insertions which were designated by the Greek letter Ω followed by an isolation number (e.g., Ω[chr::Tn551]40), 2) insertions responsible for the inactivation of a chromosomal gene (e.g., tyrB282::Tn551; Pattee, 1981), and 3) derivatives of class 2 insertions which have a point mutation within the ermB locus of Tn551 resulting in the loss of erythromycin resistance (e.g., tyrB282::Tn551 ermB321; Pattee et al., 1983). All strains were grown on BHI agar slants and stored at 4°C. For long-term storage, freshly grown cells were suspended in GL broth containing 10% (vol/vol) glycerol and stored at -70° C.

Table 1. Designation, genotype, and origin of bacterial strains

Strain	Genotype	Origin or Reference
Staphylococcus aureus		
ISP2	8325 *nov-142 pig-131*	Pattee and Neveln, 1975
ISP5	8325 *thy-101 thrB106 ilv-129 pig-131*	"
ISP7	8325 *thy-101 his-116 trp-103 pig-131*	"
ISP8	8325-4 *pig-131*	Thompson and Pattee, 1977
ISP12	8325(pI258) *thy-101 pig-131*	Lindberg et al., 1972
ISP13	8325 *trp-103 his-116 pig-131*	ISP20 DNA x ISP7[a]
ISP14	8325 *trp-103 thrB106 ilv-129 pig-131*	ISP13 DNA x ISP5
ISP20	8325 *thrB106 ilv-129 pig-131*	Pattee and Neveln, 1975
ISP39	8325 *thy-101 purA102 pig-131*	"
ISP86	8325 *uraA141 hisG15 nov-142 purA102 pig-131*	Kuhl et al., 1978
ISP92	Ps6 *tmn-3106*	Pattee, 1976
ISP95	Ps47 *tet-3490*	Stahl and Pattee, 1983a
ISP177	8325(pI258) *nov-142 pig-131*	phage 80α/ISP12 X ISP2[b]
ISP179	8325(pI258) *nov-142 tet-3490 pig-131*	ISP95 DNA x ISP177
ISP283	8325-4Ω[chr::Tn*551*]5 *tmn-3106 nov-142 pig-131*	Brown and Pattee, 1980
ISP374	8325Ω[chr::Tn*551*]40 *pig-131 tmn-3106*	"
ISP387	8325Ω[chr::Tn*551*]40 *ilv-129 pig-131 trp-103*	ISP374 DNA x ISP14
ISP479	8325-4(pI258 *blaI401 mer-14 repA36*) *pig-131*	Pattee, 1981

[a] ISP7 was transformed by DNA from ISP20.
[b] ISP2 tranduced by bacteriophage 80α lysate propagated on ISP12 (see Materials and Methods).

Table 1. (Continued)

Strain	Genotype	Origin or Reference
ISP642	8325-4 *trp-263*::Tn*551* *pig-131*	Pattee, 1981
ISP646	8325 *thy-101* *thrB106* *trp-263*::Tn*551* *ilv-129* *pig-131*	ISP642 DNA x ISP5
ISP682	8325 *thy-101* *thrB106* *tyrA283*::Tn*551* *ilv-129* *pig-131*	Stahl, 1982
ISP803	8325 Ω[*chr*::Tn*551*]42 *pig-131*	Stahl and Pattee, 1983a
ISP835	8325-4 *trp-159*::Tn*551* *ermB317* *pig-131*	Pattee et al., 1983
ISP839	8325-4 *tyrB282*::Tn*551* *ermB321*	"
ISP877	8325 *r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *tyrB282*::Tn*551* *lys-115* *trp-103* *thrB106* *ala-126* *tmn-3106* *ilv-129* *pig-131*	Stahl and Pattee, 1983b
ISP923	8325 *r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *trp-103* *thrB106* *ala-126* *tmn-3106* *ilv-129* *pig-131* *uraA141* *hisG15* *nov-142* *lys-115*	"
ISP926	8325 *r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *ala-126* *tmn-3106* *ilv-129* *pig-131* *hisG15* *nov-142* *lys-115* *trp-103* *thrB106*	"
ISP930	8325 *thrB106* *uraA141* *ilv-129* *mec-4916* *nov-142* *pig-131* *ala-126* *tmn-3106* *trpE85* *tyrB282*::Tn*551* *thy-101*	Stahl, 1982
ISP931	8325 *r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *thrB106* *ala-126* *tmn-3106* *ilv-129* *pig-131* *hisG15* *tyrA283*::Tn*551* *thy-101* *nov-142*	ISP682 DNA X ISP926

Table 1. (Continued)

Strain	Genotype	Origin or Reference
ISP932	8325 *thrB106 uraA141 ilv-129 mec-4916 nov-142 pig-131 ala-126 tmn-3106 trpE85 tyrB282::Tn551 ermB321* $\phi 12^s$	ISP839 DNA X ISP930c
ISP988	8325 *tyrB282::Tn551 ermB321 tmn-3106 ilv-129 pig-131 uraA141 nov-142 mec-4916 thrB106 trpE85*	Stahl and Pattee, 1983a
ISP1061	8325-4 *pig-131 recA1 his-7* (pI258 *blaI401 mer-14 repA36*)	phage 80α/ISP479 X RN1030
ISP1099	8325-4 *pig-131 recA1 his-7 trp-353::Tn551*	43° X 1061[d]
ISP1169	8325 *uraA141 ilv-129 mec-4916 nov-142 trpE85* Ω[*chr::Tn551*]7 *tyrB282::Tn551 ermB321 thrB106 ala-126 pig-131* $\phi 11^s$ $\phi 12^s$	RN497 DNA X ISP932
ISP1171	8325 *uraA141 ilv-129 mec-4916 nov-142 tyrB282::Tn551 ermB321 ala-126 thrB106 trpE85 tmn-3106 pig-131* $\phi 11^s$ $\phi 12^s$	ISP92 DNA X ISP1169
ISP1185	8325-4 *tyr-362::Tn551 recA1 his-7 pig-131*	ISP1061 x 43°C
ISP1236	8325 *r1⁻ r2⁻ m31⁺ m32⁺ thrB106 ala-126 ilv-129 pig-131 trp-159::Tn551 ermB317 nov-142 thy-101 tmn-3106 hisG15*	phage 80α/ISP835 X ISP931

[c] Sensitive to *S. aureus* bacteriophage φ12.

[d] Isolated by Tn*551* mutagenesis using the method of Pattee (1981).

Table 1. (Continued)

Strain	Genotype	Origin or Reference
ISP1237	8325 r1⁻ r2⁻ m31⁺ m32⁺ ala-126 tmn-3106 pig-131 hisG15 nov-142 trp-159::Tn551 ermB317 tyrB282::Tn551 ermB321 thrB106 ilv-129 nov-142	ISP839 DNA X ISP1236
ISP1238	8325 r1⁻ r2⁻ m31⁺ m32⁺ trp-103 ala-126 ilv-129 pig-131 lys-115 nov-142 tmn-3106 hisG15 ngr-374	ISP1185 DNA X ISP926
ISP1295	8325 r1⁻ r2⁻ m31⁺ m32⁺ trp-103 ala-126 ilv-129 pig-131 hisG15 Ω[chr::Tn551]40 lys-115 nov-142 ngr-374 tmn-3106	RN1855 DNA X ISP1238
ISP1297	8325-4 pig-131 thrB106	ISP5 DNA x ISP835
ISP1308	8325-4 pig-131 ngr-374 Ω[chr::Tn551]40	ISP1295 DNA x ISP1297
ISP1313	8325 r1⁻ r2⁻ m31⁺ m32⁺ ala-126 tmn-3106 pig-131 nov-142 mit-376 trp-159::Tn551 ermB317 tyrB282::Tn551 ermB321 thrB106 ilv-126	RN981 DNA X ISP1237
ISP1314	8325 r1⁻ r2⁻ m31⁺ m32⁺ ala-126 tmn-3106 pig-131 mit-376 thrB106 trp-159::Tn551 ermB317 tyrB282::Tn551 ermB321 ilv-129	RN981 DNA X ISP1237
ISP1344	8325 r1⁻ r2⁻ m31⁺ m32⁺ trp-263::Tn551 thrB106 ala-126 tmn-3106 ilv-129 hisG15 nov-142 ngr-374 lys-115 pig-131	ISP646 DNA X ISP1238

Table 1. (Continued)

Strain	Genotype	Origin or Reference
ISP1358	8325 r1⁻ r2⁻ m31⁺ m32⁺ thrB106 lys-115 tmn-3106 ilv-129 hisG15 nov-142 ala-126 ngr-374 pig-131	phage 80α/ISP2 X ISP1344
ISP1359	8325r1⁻ r2⁻ m31⁺ m32⁺ tyrB282::Tn551 thrB106 ala-126 tmn-3106 ilv-129 pig-131 nov-142 purA102 hisG15 trp-103	ISP86 DNA X ISP877
ISP1402	8325-4purA102 nov-142 pig-131 thrB106 hisG15	ISP86 DNA X ISP1297
ISP1403	8325-4purA102 nov-142 pig-131 thrB106 mit-376	ISP1314 DNA X ISP1402
ISP1404	8325-4Ω[chr::Tn551]40 purA102 nov-142 ngr-374 pig-131 hisG15	ISP86 DNA X ISP1308
ISP1405	8325-4Ω[chr::Tn551]40 mit-376 nov-142 ngr-374 pig-131	ISP1314 DNA x ISP1404
ISP1407	8325-4(ϕ12) purA102 hisG15 pig-131 nov-142	ISP86 DNA X RN740
ISP1464	8325-4(ϕ12) purA102 mit-376 pig-131 nov-142	ISP1314 DNA X ISP1407
ISP1465	8325-4(pI258) tmn-3106 nov-142 pig-131 Ω[chr::Tn551]5	phage 80α/ISP179 X ISP283
RN497	8325-4Ω[chr::Tn551]7 pig-131	Brown and Pattee, 1980
RN740	8325-4(ϕ12) pig-131	Thompson and Pattee, 1977
RN981	8325-4his-7 recA1 pig-131	Wyman et al., 1974

Table 1. (Continued)

Strain	Genotype	Origin or Reference
RN1030	8325-4(ϕ11)recA1 his-7 pig-131	Wyman et al., 1974
RN1440	8325-4his-7 mit-3 pig-131	"
RN1441	8325-4his-7 mit-6 pig-131	"
RN1855	8325Ω[chr::Tn551]40 pig-131	Pattee and Glatz, 1980
Bacillus subtilis		
A-62	W23 wild-type	IDM[e]
SB68	168trpC2 hisH2 tyrA1	BGSC[f]

[e] Received from the culture collection of the Department of Microbiology, Iowa State University, Ames, IA.

[f] Received from the Bacillus Genetic Stock Center, Ohio State University, Columbus, OH. (catalog number 1A87).

Culture Media

Brain heart infusion (BHI; Difco Laboratories, Detroit, MI), Trypticase soy broth (TSB; BBL, Cockeysville, MD) and Trypticase soy agar (TSA; BBL) were routinely supplemented with thymine (final concentration = 20 µg/ml) and adenine, guanine, cytosine, and uracil (final concentration = 5 µg/ml each). When necessary, 1.5% (wt/vol) agar (Bacto agar; Difco) was added to the medium prior to autoclaving. Soft agar was composed of 0.4% (wt/vol) agar in TSB. TSA was also enriched with $CaCl_2$ (final concentration = 5.0 mM). GL broth consisted of 3.0 g of Casamino acids (Difco), 3.0 g of yeast extract (Difco), 5.9 g of NaCl, 3.3 ml of 60% sodium lactate, and 4 ml of 95% glycerol per liter of deionized water; the final pH before autoclaving was 7.8 (Novick et al. 1979). Various formulations of complete defined synthetic (CDS) agar were prepared as described by Pattee and Neveln (1974). The composition of CDS agar is given in Table 2. The formulations of the media for selection or scoring the various markers examined in this study are given in Table 3.

Regeneration agar was used to support the formation of walled cells from protoplasts of S. aureus. This medium consisted of 30 g of dehydrated TSB, 270 g of sucrose, 25 g of Bacto-Agar, 0.5 g of sodium citrate $2H_2O$, 3.0 g of potato

Table 2. Composition of complete defined synthetic (CDS) media[a]

Ingredient	Concentration[b]	Ingredient	Concentration[b]
Basal salts:		**amino acids:**	
K_2HPO_4	7.0 gm	L-glutamate	100 mg
KH_2PO_4	3.0 gm	L-serine	30 mg
Na_3citrate$\cdot 2H_2O$	0.4 gm	L-methionine	10 mg
$MgSO_4$	0.05 gm	L-tyrosine	50 mg
$(NH_4)_2SO_4$	1.0 gm	L-alanine	60 mg
		L-lysine	50 mg
Vitamins:		L-threonine	30 mg
thiamine	1.0 mg	L-phenylalanine	40 mg
niacin	1.2 mg	L-histidine	20 mg
biotin	0.005 mg	glycine	50 mg
Ca pantothentate	0.25 mg	L-tryptophan	10 mg
		L-isoleucine	90 mg
Purines and Pyrimidines:		L-valine	80 mg
adenine	5 mg	L-leucine	90 mg
guanine	5 mg	L-aspartate	90 mg
cytosine	5 mg	L-arginine	50 mg
uracil	5 mg	L-proline	80 mg
thymine	20 mg	L-cystine	20 mg
Bacto-agar (Difco)	15 gm		
glucose	5 gm		

[a] Media prepared by combining these ingredients, except agar, and filter sterilizing. For CDS agar, the filter sterilized CDS was added to agar which was had been previously autoclaved in remaining water and cooled to 50°C.

[b] Final concentration per liter of CDS.

Table 3. Formulation of media for selecting and scoring the various markers used in this study

Marker	Phenotype	Medium used[a]
Tn*551*	Erythromycin resistance (Em^r)	BHI with a) 1 µg/ml or b) 10 µg/ml erythromycin.[b,c]
mec	Methicillin resistance (Mec^r)	TSA with 6.25 µg/ml methicillin and 5% (w/v) NaCl (Kuhl et al., 1978).[c]
nov	Novobiocin resistance (Nov^r)	BHI with 10 µg/ml novobiocin.[b,c]
tmn	Tetracycline resistance (Tmn^r)	BHI with a) 1 µg/ml or b) 10 µg/ml tetracycline.[b,c]
cad	Plasmid-borne cadmium resistance (Cad^r)	GL agar with a) 10^{-4} M $Cd(NO_3)_2$ and 0.5 gm sodium citrate$\cdot 2H_2O$ per liter, b) 5×10^{-5} M $Cd(NO_3)_2$.
ngr	N-methyl-N-nitro-N-nitrosoguanidine resistance (Ngr^r)[d]	TSA rehydrated in 0.1 M KH_2PO_4, pH 6.0 with 5 µg/ml MNNG (Goering, 1978) or TSA with 5 µg/ml MMS.[c]

[a] Media having different formulations for selection and scoring are designated with an a) and b) composition, respectively.

[b] Contains 0.5 g/liter sodium citrate$\cdot 2H_2O$.

[c] Antibiotics and DNA modifying agents added after autoclaving.

[d] Unselectable marker.

Table 3. (Continued)

Marker	Phenotype	Medium used[a]
mit	Mitomycin C resistance (Mit^r)[d]	TSA with 0.1 µg/ml Mitomycin C.
his	Histidine requiring (His^-)	CDS devoid of L-histidine.
thr	Threonine requiring (Thr^-)	CDS devoid of L-threonine.
trp	Tryptophan requiring (Trp^-)	CDS devoid of L-tryptophane.
tyr	Tyrosine requiring (Tyr^-)	CDS devoid of L-tyrosine.
Ilv	Leucine, isoleucine, and valine requiring (Ilv^-)	CDS devoid of L-isoleucine and L-leucine; L-valine reduced to 20 µg/ml plus 1% sodium pyruvate (Brown and Pattee, 1980).
pur	purine requiring (Pur^-)	CDS devoid of adenine and guanine.
ura	Uracil requiring (Ura^-)	CDS devoid of uracil and cytosine.

starch (Sigma), 5 µg/ml each of adenine, guanine, cytosine, and uracil, and sufficient deionized water to yield one liter of medium (Stahl and Pattee, 1983a).

Strains of S. aureus to be transformed were grown in CSY broth which consisted of 1.7% Casitone (Difco), 0.3% yeast extract, 0.5% NaCl, and 5.0 mM $CaCl_2$, pH 7.3 (P. A. Pattee, Iowa State University, Ames, IA; unpublished data). For transformations of Bacillus subtilis, the recipient strain was grown in SPI broth and brought to competency in SPII broth (Dubnau and Davidoff-Abelson, 1971). SPI broth was composed of Spizizen salts (0.2% $(NH_4)_2SO_4$, 1.4% K_2HPO_4, 0.6% KH_2PO_4, 0.1% sodium citrate $2H_2O$, and 0.02% $MgSO_4$ $7H_2O$; Anagnostopoulos and Spizizen, 1961) plus 0.02% Casamino acids (Difco), 0.1% yeast extract (Difco), 0.5% glucose and for auxotrophic strains 50 µg/ml of the required amino acid(s) (Dubnau and Davidoff-Abelson, 1971). SpII broth consisted of Spizizen salts plus 0.5 M $CaCl_2$, 2.5 mM $MgCl_2$, and 0.5% glucose (Dubnau and Davidoff-Abelson, 1971).

Chemicals, Reagents, and Buffers

Tetracycline (Sigma), novobiocin (Sigma), and methicillin (sodium monohydrate; Bristol Laboratories, Syracuse, NY) were prepared in deionized water (10 mg/ml), filter-sterilized, and

1-ml aliquots stored at -20°C. Erythromycin (Sigma) was prepared in 95% ethanol (10 mg/ml) and stored at -20°C. Mitomycin C (Sigma) was dissolved in sterile deionized water (1 mg/ml) and stored at 4°C and in the dark.

N-methyl-N-nitro-N-nitrosoguanidine (MNNG; Sigma; 10 mg/ml) was prepared fresh just prior to use in absolute methanol. 4-nitroquinoline oxide (NQO; Sigma; 5.0 mg/ml) was made fresh for each use in 95% ethanol. Methyl methanesulfonate (MMS; Sigma) and ethyl methanesulfonate (EMS; Sigma) were used as supplied by the manufacture.

Lysostaphin (Sigma) was dissolved at 1.0 mg/ml in a buffer consisting of 600 mg Tris(hydroxymethyl)aminomethane (Tris; Sigma), 870 mg NaCl, and 100 ml of deionized water, pH 7.5. The lysostaphin solution was filter-sterilized under positive-pressure, and 1-ml aliquots were stored at -20°C. Protease (Type XIV; Sigma; 10mg/ml) was made fresh for each use in Tris-EDTA-NaCl (0.1 M Tris, 0.15 M NaCl, and 0.1 M ethylenediamine-tetraacetic acid [EDTA], pH 7.5). Prior to use, the protease solution was allowed to self-digest for one hour at 37°C to inactivate any contaminating DNases. In later experiments, protease was prepared by dissolving 10 mg/ml in deionized water, and adjusting the pH to 5.0. This solution was held for 10 min at 80°C to inactivate contaminating

DNases, cooled, and adjusted to pH 7.0. NaCl was then added to a final concentration of 1.0 M, and aliquotes were stored at -20°C.

Sucrose-Magnesium-Tris buffer (SMTB; Stahl and Pattee, 1983a) was prepared by first dissolving $MgSO_4$ in deionized water to a final concentration of 40 mM. Tris and sucrose were then added to final concentrations of 100 mM and 800 mM, respectively, and the pH adjusted to 7.6. Tris-maleate buffer consisted of 0.1 M mono-Tris(hydroxymethyl)aminomethane maleate (Trizma-maleate; Sigma) in deionized water, pH 7.0 (Stahl and Pattee, 1983b). Ten-ml volumes of SMTB and Tris-maleate were autoclaved and stored at room temperature.

SDS-ethanol consisted of 5 gm SDS (sodium dodecylsulfate; Sigma) in 45% ethanol. Phenol for DNA extractions was distilled under N_2, saturated with 10 mM Tris, pH 8.1, and used within 72 h. As a fusogen in protoplasts fusion experiments 60% (vol/vol) polyethylene glycol (PEG-400, ave. M.W. = 400; Sigma) was used which was prepared in SMTB, autoclaved, and stored at room temperature (Stahl and Pattee, 1983a). Standard saline citrate (SSC) was composed of 6.1% NaCl and 4.4% trisodium citrate in deionized water, pH 7.2. Saline for routine dilution of cells consisted of 0.85% NaCl in deionized water.

Some modifications were made to the above buffers and reagents for experiments with B. subtilis. Tris-EDTA-NaCl for B. subtilis (Tris-EDTA-NaCl-BS), was composed of 20 mM Tris, 5.0 mM EDTA, and 100 mM NaCl, pH 7.5 (Lovett and Keggins, 1979). Protease was dissolved in Tris-EDTA-NaCl-BS and incubated at 37°C for 60 min. Hadden buffer (Hadden et al., 1983) was composed of 10% sucrose in 50 mM Tris, 15 mM dithiothreitol (Sigma), 200 mM NaCl, and 1 mM phenylmethanesulfonyl flouride (Sigma), pH 8.0. Bioassays were run in HEPES buffer consisting of 100 mM HEPES ([N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid]-KOH; Nutritional Biochem. Corp., Cleveland, OH), and 3.0 mM $MgCl_2$, pH 8.5 (Ljundquist et al., 1976).

For radioisotopic studies, a scintillation cocktail consisting of 0.6% (wt/vol) 2,5-diphenyloxazole (Beckman Instruments Inc., Fullerton, CA), 0.01% (wt/vol) 1,4-bis [2-(5-phenyloxazole)]benzene (Sigma) in a Triton-X100-toluene solution was used (K. D. Spence, Washington State University, Pullman, WA; unpublished data). To prepare the cocktail, PPO and POPOP were first dissolved in two parts toluene followed by the addition of one part Triton-X100 (Sigma).

Isolation of Standard Transforming DNA

Standard transforming DNA was prepared by a modified version of the protocol described by Pattee and Neveln (1975). Cells from which DNA was to be extracted were grown overnight at 35°C in 100 ml of BHI with shaking. The cells were harvested at 10,000 x g for 10 min at 4°C, washed once in 10 ml of saline, and suspended in 5 ml of Tris-EDTA-NaCl. These cells were transferred to a 50-ml screw-capped erlenmeyer flask, 0.3 ml of lysostaphin was added, and the flasks incubated for 30 min in a 35°C waterbath with shaking (ca. 110 cycles/min). Protease (1 ml) was added, and the lysate was incubated stagnant for 60 min at 35°C. SDS-ethanol (0.6 ml) was then added and the cell-lysate was shaken vigorously on a Burrell wrist-action shaker (Burrell Corp., Pittsburgh, PA) for 30 min at room temperature. After the SDS-ethanol treatment, the lysate was extracted with an equal volume of phenol (ca. 6 ml) on a rotary platform with a horizontal shaft at approximately 60 rpm for 30 min at room temperature and then centrifugated at 10,000 x g at 4°C for 45 min. The aqueous (upper) layer was removed with a pipet and extracted with phenol as before. After the second extraction, the aqueous phase was transferred to a 50-ml Erlenmeyer flask, one-tenth volume of 3.0 M sodium acetate was added, the

contents of the flask were mixed, and two volumes of cold (-20°C) 95% ethanol were added. The precipitated DNA was then spooled onto a glass rod, transferred to fresh 95% ethanol, and stored overnight at 4°C. The DNA was then aseptically transferred to a tube of sterile SSC and kept at 4°C for at least 48 h before use.

Isolation of High Molecular Weight DNA

The isolation of high MW DNA was as described by Stahl and Pattee (1983b). Cells from which high MW DNA was to be isolated were grown overnight in 100 ml of TSB at 35°C with shaking. Cells were harvested and washed in saline as described for the isolation of standard transforming DNA. The washed cells were suspended in 10 ml of SMTB and transferred to a 50-ml screw-capped erlenmeyer flask. These cells were converted to protoplasts by the addition of lysostaphin (0.3 ml) followed by a 45-min incubation at 35°C on a rotary platform (ca. 36 rpm). The protoplasts were pelleted (3400 x g for 10 min at room temperature) and gently lysed in 5 ml of Tris-EDTA-NaCl. The lysate was transferred to a 50-ml screw-capped Erlenmeyer flask, 1.0 ml of protease was added, the contents were mixed, and held at 35°C for 60 min. After 60 min, 0.6 ml of SDS-ethanol was added and the lysate was

shaken vigorously by hand for 1 min. Six milliliters of phenol were added and the lysate was extracted on a rotary platform for 5 min (ca. 20 rpm). The lysate was centrifuged at 10,000 x g at 4°C for 60 min and the aqueous layer transferred to a 50 ml screw-capped Erlenmeyer flask. The DNA was precipitated and stored as previously described for standard transforming DNA.

Isolation of S. aureus Cell-Free Extracts

Cell-free extracts from S. aureus were prepared from 100 ml of mid- to late-log phase cells (OD_{540nm} = 0.65) grown in TSB at 35°C. The cells were harvested, washed once with saline, resupended in 10 ml of SMTB, and converted to protoplasts as previously outlined for the preparation high MW DNA. The protoplasts were pelleted by centrifugation at 3500 x g for 5 min at room temperature and resuspended in 2 ml of Hadden buffer. The protoplast suspension was place on ice and Brij 58 (Sigma) was added to a final concentration of 0.2% (wt/vol). The suspension was incubated at 35°C for 3 min to lyse the protoplasts and then centrifuged at 30,000 x g for 60 min in a SW-60 rotor (Beckman Instruments, Palo Alto, CA). After centrifugation, 1-ml samples of the supernatant (cell-free extract) were stored at -70°C. The protein

concentration in the cell-free extracts was determined by a modified version of the colorimetric assay described by Lowry et al. (1951). To 0.2 ml of cell-free extract, 0.05 ml of 1.5 N NaOH was added and the mixture was heated in a boiling water bath. After 10 min, the extracts were cooled to room temperature and 2.5 ml of freshly prepared 0.5% (wt/vol) $CuSO_4$ in 2% Na_2CO_3 was added. The extracts were incubated for 10 min at room temperature, and 0.25 ml of 1 N Folin reagent (Fisher Scientific Co., Fairlawn, NJ) was added. The assay developed maximum color after 30 min at room temperature and the OD was read at 623nm (R. E. Hurleburt, Washington State University, Pullman, WA; personal communication). Bovine serum albumin (Fraction V, Nutritional Biochemical Co.) was used to construct the standard protein curve.

Propagation of S. aureus Bacteriophage

S. aureus bacteriophages 80α and 83A were obtained from Dr. P. A. Pattee (Iowa State University, Ames, Iowa) and stocks of these bacteriophages were maintained by propagation on ISP8 and ISP201, respectively. Propagation of bacteriophage as stocks or transducing lysates were exactly the same, and followed the procedure of Pattee and Baldwin (1962). BHI agar slants (18mm x 150mm) of the appropriate S.

aureus strains were grown overnight at 35°C. The cells were harvested from each slant with 1 ml of TSB. To a tube containing 4 ml of melted soft agar at 45°C, 0.1 ml of the cell suspension was added followed by 0.05 ml of bacteriophage stock (titer = 10^{10} to 10^{11} plaque-forming units (PFU)/ml). The contents of the tube were mixed and poured onto the surface of a fresh TSA plate. The plates were incubated at 35°C for 4 to 6 h, or until clearing of the soft agar overlay was apparent. The soft agar overlay was then homogenized in 10 ml of TSB with a 10 ml pipet and the homogenate centrifuged for 20 min at 3400 x g at room temperature. The supernatant was sterilized by passage through an 02 Selas filter (Selas Corporation of America, Spring House, PA), transferred aseptically to sterile screw-capped tubes, and stored at 4°C. All bacteriophage lysates were titered on the strain of S. aureus on which they were propagated and lysates containing between 10^{10} and 10^{11} PFU/ml were used in transductions.

Transduction of S. aureus

Transductions of S. aureus were performed using the procedure described by Kasatiya and Baldwin (1967), as modified by Schroeder and Pattee (1984). Recipient cells were grown overnight on BHI agar slants (18mm x 150mm). The cells

on each slant were harvested into 1 ml of TSB, and 0.5-ml samples (containing between 1 and 5 x 10^{10} cells) were transferred to two centrifuge tubes. Sufficient transducing lysate (usually 0.25 to 0.5 ml) was added to the experimental tube to obtain a multiplicity of infection of approximately 1.0. Both the experimental and the control tubes were brought to a final volume of 2 ml with 5 mM $CaCl_2$ in TSB and incubated in a 35°C water bath with vigorous shaking. After 20 min, 1 ml of ice-cold 0.02 M sodium citrate was added to each tube and the tubes were centrifuged at 3400 x g for 7 min. The cells were resuspended in 1 ml of cold 0.02 M sodium citrate, and 0.1-ml samples were spread onto the appropriate selective media.

Genetic Transformation of S. aureus

S. aureus was transformed as described by Stahl and Pattee (1983b). Recipient strains were grown overnight on BHI agar slants (18mm x150mm). The cells were resuspended in 5 ml of saline, and this suspension was used to adjust a 300-ml nephelometer flask containing 100 ml of CSY broth to an OD_{540nm} of 0.2. Ten-milliliter samples were then transferred to six 100-ml volumes of CSY broth, which were incubated in a 35°C water bath with shaking (ca. 110 cycles/min). When an

OD_{540nm} of 0.2 was reached, the cells were harvested by centrifugation (10,000 x g for 30 min, 4°C) and resuspended in 1 ml of TSB. The cells were transferred to a sterile centrifuge tube containing 1 ml of normal rabbit serum (Pel-Freez Biologicals, Rogers, AZ) and 1.2 ml of S. aureus bacteriophage ϕ55 (Thompson and Pattee, 1977). The cell suspension was incubated for 5 min in a 35°C water bath with shaking. The cells were then pelleted (3500 x g, 7 min), washed once with Tris-maleate, and resuspended in 6.0 ml of Tris-maleate buffer containing 0.1 M $CaCl_2$. One-milliliter aliquots of these cells were then added to each of 5 centrifuge tubes containing 0.2 ml of transforming DNA, and one centrifuge tube without DNA (i.e., control tube). The contents of the tubes were mixed, held at 4°C for 2.5 min, transferred to 35°C for an additional 2.5 min, and then centrifugated at 3500 X g for 7 min. Each pellet was gently resuspended with 1 ml of BHI containing 2.0 mM sodium citrate and incubated at 35°C with gentle shaking. After 60 min, the cells were pelleted, resuspended in saline, and plated onto the appropriate selection medium. Colony forming units (CFU) were determined from the control cells on BHI agar plates. When desired, the occurrence of unselected markers among the transformants was scored by replica plating the transformants

onto the appropriate scoring media.

Protoplast Fusion Analysis

The protocol for protoplast fusions and the analysis of the recombinants resulting from protoplast fusion was as described by Stahl and Pattee (1983a). The parental strains for these experiments were grown overnight on BHI agar slants and suspended in saline as previously described for transformation experiments. Each saline suspension of parental cells was used to inoculate a 300-ml nephelometer flask containing 100 ml of TSB to an initial to OD_{540nm} of 0.1. These cultures were incubated at 35°C with shaking until the OD_{540nm} was about 0.65. The cells were then harvested at 10,000 x g for 10 min. To insure that the parental strains could be harvested simultaneously, the starting time of each 100-ml culture was adjusted accordingly. Each of the parental strains was washed once with saline and resuspended in 10 ml of SMTB. Protoplasts were formed from these cells by the protocol outlined for the isolation of high MW DNA.

After lysostaphin treatment, the protoplasts were centrifuged for 10 min at 3500 x g, and resuspended in 1 ml of SMTB. One-tenth milliliter of protoplasts from each parental strain was added to a sterile test tube, followed by 1.8 ml of

PEG-400. The protoplasts were incubated for 1 min at 20°C, serially diluted (10-fold dilutions) in SMTB, and 0.05-ml samples were spread onto regeneration agar. The regeneration agar plates were incubated at 35°C in a humidified incubator for between 5 and 7 days.

The cells from the regeneration agar plates were harvested with 10 ml of TSB. These cells were sonicated for 1 min (20 Kcp, Biosonik II sonicator, Bronwill Scientific, Rochester, NY), and sterile glycerol was added to a final concentration of 10% (vol/vol). The number of recombinants per ml was estimated by plating 0.1-ml samples of serially diluted cells onto media selective for one marker from each parental strain. The remaining cell suspension was stored at -70°C until the approximate number of recombinants per ml was determined.

When the approximate number of recombinants per ml was known, the cell suspension was thawed and adjusted with saline to contain approximately 2×10^3 recombinants per ml. Samples (0.1 ml) were plated onto the appropriate selective media and incubated at 35°C until the recombinants were of sufficient size to be counted. Individual recombinants were then transferred to ten plates of fresh selection medium, each plate receiving 56 recombinants arranged in a uniformed pattern. These plates were incubated for 24 h at 35°C, and

then velveteen-replicated onto a fresh plate of selective medium and incubated at 35°C for 24 h. These replicas were then velveteen-replicated to various media to score the occurrence of the unselected markers among the recombinants. After 24 h, the contaminants and missing colonies were clearly marked on each of the replica plates, and the recombinants were analyzed with the aid of a programmed computer (CMB 8032 personal computer, model 8050 dual disk drive, and a model 8300P printer; Commodore Business Machines, Inc., West Chester, PA). Briefly, the growth response of each recombinant on the various media was recorded, a matrix formed, and the coinheritance frequencies for the markers were determined. The coinheritance frequency (CIF) for a specific pair of markers was defined as the percentage of the total recombinants that exhibited the phenotypes characteristic to one or the other parent for that specific pair of markers (Stahl and Pattee, 1983a).

Inactivation Assays with DNA-Modifying Agents

The sensitivity of S. aureus to DNA-modifying agents was determined using inactivation assays. The protocols for the inactivation assays were generally the same for all assays. Strains of S. aureus to be assayed were grown overnight at 35°C on BHI slants. The cells were suspended in 5 ml of

saline and used to inoculate the various assay media to an OD_{540nm} of about 0.2 (ca. 10^7 to 10^8 CFU/ml). All assays were incubated at 35°C; samples were removed at regular intervals, serially diluted (10-fold dilution) in saline, and plated onto BHI agar. Survivors were determined after 24 h at 35°C.

Assays with MNNG and MMS were performed in 0.85% (wt/vol) sodium citrate, pH 5.0 and TSB, respectively. Nitrous acid sensitivity was assayed in a modified form of the assay medium described by Karren et al. (1980). This medium contained 4.75 ml TSB and 0.25 ml of freshly prepared 0.2 M $NaNO_2$ in 0.1 M sodium acetate, pH 4.6. UV sensitivity was assayed as described by Goering and Pattee (1971). Cells to be UV irradiated were suspended in saline and a 15-watt germicidal lamp (Sylvania Electric Prod., Dansver, MD) was used as a source of UV radiation.

Disk Assays

A quick, semi-quantitative assay for sensitivity of S. aureus to various agents was developed by modifying the disk assay of Ljundquist et al. (1976). Cells to be assayed were grown and harvested as previously described for the inactivation assays. Each strain of S. aureus was adjusted to an OD_{540nm} of 1.0 in saline, and 0.25-ml samples of these

suspensions were used to inoculate 4-ml volumes of melted soft agar. Each tube of soft agar was poured onto the surface of a TSA plate and allowed to solidify at room temperature. After at least 4 hours at room temperature, two sterile filter-paper disks (0.5 inches in diameter; Schleicher and Schuell, Keene, NH) were placed on the surface of each plate and 20 µl of test agent was applied to each disk. The plates were incubated at 35°C overnight, after which the diameters of the zones of inhibition were measured. These diameters were proportional to the sensitivity of the strain to the tested agent.

DNA Degradation Assays

The degradation of cellular DNA after UV irradiation of S. aureus was assayed by using a modification of the assay described by Goering (1979). Cells to be assayed were grown overnight in 2 ml of BHI at 35°C with shaking. The next morning, 0.2 ml of these cells were transferred to 3 ml of BHI containing 0.1 ml of [^{3}H]methyl-thymidine (Sp. Act. = 6.7 Ci/mmole; Amersham, Arlington Heights, IL) and adenine, guanine, cytosine, and uracil (5 µg/ml each). The cells were incubated at 37°C with shaking for at least two generations (ca. 4 h), after which the cells were pelleted at 3500 x g for 7 min and washed twice in saline. The washed cells were

resuspended in 3 ml of BHI supplemented with adenine, guanine, cytosine, and uracil (5.0 µg/ml each), and incubated for an additional 60 min at 37°C. The cells were then pelleted, resuspended in 3 ml of saline, and split into two 1.5-ml samples. One of these samples received 6.0 J/M^2 of UV radiation from a 15-watt germicidal lamp. The control and UV irradiated samples were diluted 1:10 with BHI supplemented with 100 µg/ml thymidine and incubated at 35°C. After 3 h, the amount of radiolabel released due to degradation was determined by transferring a 0.5-ml aliquot from each sample to separate 1.5-ml micro-test tubes (Brinkman Instruments, Westbury, NY), adding 0.5 ml of 13.3% (wt/vol) trichloroacetate (TCA) in deionized water and placing these tubes on ice for 2 h. The total amount of [^{3}H]methyl-thymidine incorporated into the DNA was determined by transferring a second 0.05-ml aliquot from each sample into separate micro-test tubes, adding 0.5 ml of TCA, and heating the tubes at 85°C for 60 min to completely hydrolysize the DNA (Goering, 1979). After heating, the tubes were placed on ice for 2 h. All tubes then received 0.1 ml of 1.0% bovine serum albumin (Nutritional Biochemicals, Inc.) in deionized water, and were centrifuged for 10 min at 10,000 x g. A 0.9-ml sample of the supernatant from each tube was removed, added to

10 ml of scintillation cocktail, and the counts per min determined with a 1217 Rackbeta scintillation counter (LKB Wallac, Turku, Finland). The percent of DNA degraded was determined by dividing the amount of label released by the total label incorporated into the DNA of each sample.

AP Endonuclease Bioassays with B. subtilis

Bioassays for AP endonuclease were conducted as described by Ljundquist et al. (1976). DNA from A-62 (see Table 1) was purified as described by Lovett and Keggins (1979). A-62 cells were grown overnight in Penassay broth (Difco) at 35°C with shaking. Ten milliliters of this culture were removed, centrifuged (3500 x g for 10 min), and the cells washed twice in Tris-EDTA-NaCl-BS buffer. The cells were resuspended in 2.5 ml of Tris-EDTA-NaCl-BS buffer containing 500 µg/ml of lysozyme (Sigma) and incubated for 60 min at 35°C. An equal volume of Tris-EDTA-NaCl-BS was then added, followed by the addition of protease in Tris-EDTA-NaCl-BS to a final concentration of 500 µg/ml and Sarkosyl (ICN K&K, Plainsview, NY) to a final concentration of 0.8% (wt/vol). This mixture was then incubated for 30 min at 37°C to lyse the cells. The lysate was extracted three times with equal volumes of phenol as described for other DNA preparations, except the lysate was

centrifuged at 3500 x g for 20 min at room temperature rather than at 10,000 x g for 60 min at 4°C. Precipitation of the DNA was also as previously described for the isolation of standard transforming DNA from S. aureus and the precipitated DNA dissolved in SSC, pH 5.0.

For transformation, SB68 cells were brought to competency as described by Dubnau and Davidoff-Abelson (1971). Ten milliliters of SPI broth was inoculated with SB68 and grown overnight at 30°C with shaking. This starter culture was used to inoculate 100 ml of fresh SPI broth to an OD_{540nm} of 0.05 and incubated at 37°C with shaking. When the cells entered stationary phase, 10 ml of cells were transferred to 100 ml of SPII broth and the incubation continued for 90 min with very gentle shaking. After 90 min, the cells were centrifuged at 10,000 x g at 4°C for 10 min and 100 ml of supernatant were aseptically transferred to a sterile flask containing 10 ml of sterile glycerol. Ten milliliters of this solution was used to resuspend every 100 ml of competent cells. The cells were then dispensed into sterile tubes and stored at -70°C.

To assay the AP endonucleolytic activity of S. aureus cell-free extracts, DNA from A-62 was dissolved (19 µg/ml) in SSC, pH 5.0, and depurinated by incubation for 110 min at 70°C. The cell-free extracts were adjusted to the desired

protein concentrations with Hadden buffer and 5 µl of each added to 100 µl of HEPES buffer. The assay was started by the addition of 20 µl of depurinated DNA and then allowed to run for 15 min at 37°C. The assay was stopped by the addition of 5 µl of 0.2 M EDTA, pH 7.0, followed by a 3-min incubation at 70°C. Competent SB68 cells, stored at -70°C, were quickly thawed in a 37°C waterbath and 0.5 ml of these cells were added to each assay mixture. These assay mixtures were incubated for 30 min at 35°C, 0.1 ml of each assay mixture was then spread onto CDS agar devoid of tryptophan, and incubated over night at 37°C. The AP endonuclease activity in the cell-free extracts was inversely proportional to the transformation activity of the depurinated DNA treated with the cell-free extract. The transformation activity of the depurinated DNA was directly proportional to the percent of Trp^+ transformants. The percent of Trp^+ transformants were calculated from the number of Trp^+ transformants recovered from depurinated DNA treated with cell-free extract divided by the number of Trp^+ transformants recovered from the control DNA (i.e., depurinated DNA not treated with the cell-free extract).

RESULTS

Preliminary Observations

The recombination-deficient strains of S. aureus used throughout this study carried the recA1 mutation described by Wyman et al. (1974). Strains carrying recA1 were deficient in recombination, prophage induction, and DNA modification (Mod^-; Wyman et al., 1974), sensitive to UV irradiation and MitC, and exhibited lethal sectoring, with only 10% of the population being viable. Goering (1979) also showed that RN981, which carries recA1, was sensitive to MNNG and degraded its DNA when UV irradiated.

Given these phenotypes, various media were formulated in order to find one capable of scoring recA1. For scoring the recA1 mutation, the best medium was MNNG agar (Table 4). On this medium, RN981 and RN1030, recA1-carrying strains, failed to grow at concentrations of MNNG greater than 5 µg/ml. Rec^+ strains, with the exception of RN1441, were unaffected by even 10 µg/ml of MNNG (Table 4). RN1441, was isolated as a spontaneously occurring Rec^+ revertant of RN981 on a MitC-containing medium by Wyman et al. (1974). While RN1441 exhibits most of the phenotypes associated with Rec^+ strains,

Table 4. Sensitivity of various strains of S. aureus 8325 to MNNG, MitC, and MMS[a]

Strain	Rec phenotype	Minimum concentration at which no growth occurred[b]:		
		MNNG	MitC	MMS
ISP2	+	>10 µg/ml	0.05 µg/ml	>10 mM
ISP8	+	>10 µg/ml	>0.2 µg/ml	>10 mM
ISP988	+	>10 µg/ml	0.05 µg/ml	5 mM
RN981	-	5 µg/ml	0.05 µg/ml	5 mM
RN1030	-	5 µg/ml	0.03 µg/ml	5 mM
RN1441	+	10 µg/ml	>0.1 µg/ml	5 mM

[a] For composition of these media see Table 3.

[b] MNNG = N-methyl-N-nitro-N-nitrosoguanidine; MitC = mitomycin C; MMS = methylmethanesulfonate.

it is still sensitive to UV irradiation and MNNG. RN1441 and a strain with a similar phenotype, RN1440, are not considered to be true revertants of recA1, but rather partial-revertants carrying the point mutations mit-6 and mit-3, respectively, (Wyman et al., 1974).

RN1441 and ISP988 showed the same level of sensitivity to MMS as the recA1 strains (Table 4). The sensitivity of ISP988, a multiple lysogen, to MMS was attributed to the induction of prophage (Kondo et al., 1970). The sensitivity of RN1441 to MMS further supported the conclusion of Wyman et al. (1974), that this strain was not a true revertant. ISP2 and ISP988 were sensitive to the same concentration of MitC as RN981 and RN1030. In contrast, the nonlysogenic strains ISP8 and RN1441 were resistant to fairly high concentrations (Table 4). It appeared, therefore, that MitC was causing prophage induction in ISP2 and ISP988. In general, of the media examined, MNNG agar was applicable in scoring both lysogenic and nonlysogenic strains for recA1. However, because MitC and MMS agars were stable during extended incubations at 37°C, these agents were favored over MNNG in scoring nonlysogenic strains of S. aureus.

The Mod$^-$ phenotype of recA1 strains (Wyman et al., 1974) was confirmed by propagating bacteriophage 80α on recA1

strains and their derivatives, and determining the efficiency of plating of these bacteriophage determined on the Rec$^+$ strains ISP8, ISP923 and ISP926. ISP923 and ISP926 are deficient in DNA restriction. In all cases, bacteriophage propagated on strains which either carried recA1 or were derived from such strains (i.e., RN1440 and RN1441) had plating efficiencies less than 1.0% on ISP8 (Table 5). In contrast, the plating efficiencies on ISP923 or ISP926 were at least 10- to 100-fold greater than on ISP8. These results confirmed that the recA1 mutants were Mod$^-$, but also showed, contrary to a previous report (Wyman et al., 1974), that both RN1440 and RN1441 were Mod$^-$. Furthermore, these results indicated that restrictionless strains such as ISP923 and ISP926 would have to be used as recipients in mapping experiments using DNA isolated from recA1 mutants or their revertants. However, bacteriophage 80α propagated on ISP8 gave the same plating efficiencies on RN1030 and ISP1099 as on ISP8 (Table 5). The latter results indicated that the recA1 mutants were restrictionless.

Identification of the ngr-374 Allele

The approach used in mapping the recA1 allele was essentially as outlined by Stahl and Pattee (1983b).

Table 5. Efficiency of plating (EOP) for bacteriophage 80α propagated on various strains of S. aureus

Propagating strain	Rec phenotype	Indicator strain	EOP[a]
ISP8	+	ISP8	1.0
		RN1030	1.0
		ISP1099	1.0
ISP1099	-	ISP1099	1.0
		ISP923	0.1
		ISP8	0.004
ISP1185	-	ISP1185	1.0
		ISP923	0.1
		ISP8	0.001
RN1030	-	RN1030	1.0
		ISP926	0.1
RN1440	+	RN1440	1.0
		ISP8	0.005
RN1441	+	RN1441	1.0
		ISP8	0.005

[a] EOP reported as the fraction of PFU/ml recovered from the test strains compared to the PFU/ml recovered from the propagating strain.

Generally, protoplast fusion analysis was used to predict the linkage of the allele responsible for the Rec$^-$ phenotype in the recA1 mutants to known chromosomal markers, with confirmation of the predicted linkage by transformation. For protoplast fusion analysis, the Rec$^+$ parent was ISP1171 (restriction-proficient). This choice was made in spite of the Mod$^-$ phenotype of the recA1 parent, and was based on a previous report that the transposon Tn916 was transferred to S. aureus by protoplast fusion between S. aureus and Streptococcus faecalis (personal communication, P. A. Pattee, Iowa State University, Ames, IA). It was assumed that if such a protoplast fusion was successful then perhaps restriction-modification patterns were not important in protoplast fusion experiments. In addition, MNNG sensitivity (i.e., Ngr^S) was selected to score the recA1 allele; since ISP1171 lacked all prophage except ϕ13, the likelihood that MNNG induction of prophage would bemistaken for Ngr^S (i.e., Rec$^-$) was minimized.

Protoplasts of ISP1185 and ISP1171 were fused, and Em^r Nov^r and Em^r Tmn^r recombinants were selected from the regenerated population. Both selections resulted in similar numbers of recombinants (ca. 3.0×10^5 CFU/ml), as shown in Table 6. The values shown are the CIFs for the recombinants and are used to predict the probability of linkage between a

Table 6. Coinheritance frequencies for an ISP1185 x ISP1171 protoplast fusion[a]

Coinheritance frequencies (%) of Em^r Nov^r recombinants[b]

	Thr	Trp	Ngr	Em	Tmn	Ilv	Ura	Nov	Mec
Thr	100	47	78	91	9	11	11	9	10
Trp	47	100	40	40	60	61	62	60	57
Ngr	78	40	100	81	19	19	17	19	21
Em	91	40	81	100	0	2	2	0	4
Tmn	9	60	19	0	100	98	98	100	96
Ilv	11	61	19	2	98	100	97	98	94
Ura	11	62	17	2	98	97	100	98	94
Nov	9	60	19	0	100	98	98	100	96
Mec	10	57	21	4	96	94	94	96	100

Coinheritance frequencies (%) of Em^r Tmn^r recombinants[b]

	Thr	Trp	Ngr	Em	Tmn	Ilv	Ura	Nov	Mec
Thr	100	60	73	92	8	22	27	9	93
Trp	60	100	41	58	42	53	58	40	60
Ngr	73	41	100	77	23	25	21	26	74
Em	92	58	77	100	0	16	19	4	96
Tmn	8	42	23	0	100	84	81	96	4
Ilv	22	53	25	16	84	100	83	83	18
Ura	27	58	21	19	81	83	100	77	22
Nov	9	40	26	4	96	83	77	100	4
Mec	93	60	74	96	4	18	22	4	100

[a] ISP1185 = Thr^+ Em^r Ngr^s Trp^+ Tmn^s Ilv^+ Ura^+ Mec^s Nov^s; ISP1171 = Thr^- Em^s Ngr^r Trp^- Tmn^r Ilv^- Ura^- Mec^r Nov^r $\phi 11^s$ $\phi 12^s$. Em^r = tyr362::Tn551.

[b] The numbers represent the percentages of the total number of recombinants with either of the parental phenotypes for the indicated pairs of markers (Stahl and Pattee, 1983a).

pair of markers (Stahl and Pattee, 1983a; Stahl and Pattee, 1983b). Generally, a high CIF between a pair of markers was taken as an indication of possible linkage. Among the Em^r Nov^r recombinants, Ngr showed a high CIF with Thr (78%) and Em (81%). These results suggested that the locus for Ngr^s was linked to thrB106 and tyr-362::Tn551 in linkage group I. Included in the thrB106 - tyr362::Tn551 linkage group is trpE85 which is known to be linked to thrB106 and tyrA281::Tn551 by transformation (Pattee, 1981). However, the CIF between Ngr and Trp was 40% or approximately half the CIF between Ngr and either Thr or Em and less than expected if the Ngr locus was linked to the thrB106 - trpE85 - tyr-362::Tn551 linkage group. However, the CIFs between Thr and Trp (47%), and Em and Trp (40%) were nearly identical to that of Ngr and Trp, and also lower than expected between linked loci. Stahl (1982) reported similar results between trpE85 and tyrB282::Tn551 ermB321 and suggested that this could be a region of the chromosome which experienced a high frequency of recombination. In view of this, the low CIFs observed between Trp and Thr, Ngr, and Em were discounted.

When the Em^r Tmn^r recombinants were analyzed, Ngr exhibited high CIFs with Thr (73%) and Em (77%) while Trp showed reduced CIFs with these markers (Table 6). Moreover,

possible linkage between Mec and Ngr was detected among these recombinants. The CIF between this pair of markers was equivalent to those observed between Ngr and either Thr or Em (Table 6). These results, except for the proposed linkage between Mec and Ngr, were in agreement with the results reported for the Em^r Nov^r recombinants. Based on these data, it was concluded that the Ngr locus was probably linked to the linkage group thrB106 - trpE85 - tyr-362::Tn551, but between thrB106 and mec-4916 (see Fig. 6). Based on phenotype and evidence to be presented showing that the Ngr^s allele was not involved in genetic recombination, this allele was designated ngr-374.

Confirmation of the predicted linkage of ngr-374 to thrB106 was accomplished by transforming ISP926 with high MW DNA from ISP1185. In this experiment, the transformants were doubly selected for either Thr^+ Trp^+ or Em^r Trp^+. The rationale for these selections was that if ngr-374 was within the thrB106 - trpE85 - tyr-362::Tn551 linkage group then these selections should increase the probability of isolating this mutation. Among the Thr^+ Trp^+ transformants, 87% were Ngr^s Em^r and 13% were Ngr^r Em^r (Table 7). These results suggested that ngr-374 was either to the right of tyr-362::Tn551 or to the left of thrB106 (see Fig. 6). Among the Trp^+ Em^r

Table 7. Transformation of ISP926 with DNA from strain ISP1185[a]

	Selection for:	
Frequency[b] of:	Thr^+ Trp^+	Em^r Trp^+
Transformation	159	377
Reversion	<10	<10

	Relevant markers			
	Ngr	Thr	Trp	Em
Donor:	s	+	+	r
Recipient:	r	-	-	s
Analysis of transformants selected for:[c]				
Thr^+ Trp^+ (189)				
87.0%	s	+	+	r
13.0%	r	+	+	r
Em^r Trp^+ (383)				
77.0%	r	-	+	r
22.0%	r	+	+	r
0.7%	s	+	+	r

[a] ISP926 = 8325r1⁻ r2⁻ m31⁺ m32⁺ lys-115 trp-103 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142; ISP1185 = 8325-4pig-131 tyr-362::Tn551 recA1 his-7.

[b] Expressed as colonies per 10^9 CFU; 1.2 x 10^9 CFU per ml of transformation suspension and an excess of transforming DNA was used.

[c] Numbers within the parentheses are the numbers of transformants examined.

transformants, ngr-374 was coinherited in only 0.7% of the transformants and all were Thr$^+$ (Table 7). These results suggested the probable gene order of these markers was ngr-374 - thrB106 - trpE85 - tyr-362::Tn551. If this gene order was correct, then ngr-374 should be cotransformable with thrB. To test this possibility, ISP926 was transformed with high MW DNA from ISP1185 with selection for Thr$^+$ transformants. Among the Thr$^+$ transformants, 13% were Ngr^s; one of these transformants was retained as ISP1238.

The recombination proficiency of ISP1238 was evaluated by transduction using bacteriophage 83A propagated on ISP1465, which carries pI258. The premise for this evaluation was that transduction and expression of a plasmid-borne marker (e.g., Cad^r) should be independent of the recombination functions of the cell, while expression of transduced chromosomal markers should be dependent on these functions (Wyman et al., 1974; Goering, 1979). Also, in Rec$^+$ cells a chromosomal marker should be transduced at a frequency that is reproducible, and proportional to the transduction frequency of the plasmid-borne marker. Therefore, recombination proficiency of a strain could be reported as the ratio of the transduction frequency of a chromosomal-borne marker to the transduction frequency of a plasmid-borne marker (CP ratio). By comparing

the CP ratios of a presumptive Rec$^-$ mutant to those of an isogenic Rec$^+$ strain an indication of the level of recombination proficiency should be obtained. To adjust for differences in the frequencies of transduction for pI258 which occurred, even in isogenic strains (e.g., see Table 8), a correction factor was introduced to normalized the data. The correction factor was based on the assumption that regardless of the recombination proficiency, the only parameter anticipated to affect the transduction frequency of a plasmid would be the incidence of nonviable cells in the recipient population. Therefore, any significant differences in transduction frequencies of pI258 into a presumptive Rec$^-$ mutant and an isogenic Rec$^+$ strains would reflect differences in cell viabilities. The correction factor was calculated on this basis and was used to normalize the transduction frequency of pI258 in the presumptive Rec$^-$ mutant. Because the transduction frequencies of the chromosomal markers should be proportional to the transduction frequency of pI258, the transduction frequencies of the chromosomal markers in the presumptive Rec$^-$ mutants were also normalized with this correction factor.

Table 8 shows the results of an evaluation of the recombination proficiency of the ngr-374-carrying mutant

Table 8. Analysis of recombination proficiency of ISP926 and ISP1238 by transduction with bacteriophage 83A/ISP1465

Strain	Ngr phenotype	Transduction frequency[a]	Correction factor[b]	CP ratio[c]
ISP926	r	Cad^r = 4.3	-	
		His^+ = 3.9	-	0.9
		Trp^+ = 7.9	-	1.8
ISP1238	s	Cad^r = 2.7	1.6	
		His^+ = 2.9	"	1.1 (120%)
		Trp^+ = 4.0	"	1.5 (83%)

[a] The Cad^r determinant is on pI258, while the His^+ and Trp^+ determinants are chromosomal. Frequencies are expressed as transductants recovered per 10^6 CFU.

[b] Calculated by dividing the Cad^r transduction frequency for Rec^+ strain (i.e., ISP926) by the Cad^r transduction frequency for presumptive Rec^- mutant (i.e., ISP1238) and used to normalized the transduction frequency of the presumptive Rec^- mutant to that of the Rec^+ strain.

[c] Ratio of the transduction frequency of the chromosomal markers to the transduction frequency of the plasmid marker (Cad^r). The values in parentheses are the percent of the Rec^+ strain CP ratio exhibited by the presumptive Rec^- mutant.

ISP1238 and ISP926. The correction factor for ISP1238 transductants was calculated by dividing the Cad^r transduction frequency in the wild-type strain ISP926 by the Cad^r transduction frequency in ISP1238. The correction factor for ISP1238 was 1.6; the transduction frequencies obtained with ISP1238 were normalized before calculating the CP ratio (Table 8). When the CP ratios of ISP926 and ISP1238 were compared, there was no significant difference between them. Apparently, ngr-374 in the ISP926 background does not affect the recombinational activity.

There are three explanations for these results. First, ngr-374 may not be involved in recombination, but may be another mutation effecting DNA repair of MNNG-generated lesions. Second, the Rec^- phenotype in S. aureus might be polygenic and ngr-374 is but one of at least two required mutations. Finally, since ISP926 is lysogenic for ϕ11, ϕ12, and ϕ13, it is possible that one or more of these prophage may be complementing any effect ngr-374 has on recombination (Wyman et al., 1974; Reyes, 1982). The latter possibility was the simplist to test because of the availability of nonlysogenic derivatives of S. aureus 8325.

A derivative of ISP1238, ISP1295 (Table 1), was constructed by introducing Ω[chr::Tn551]40 (Ω40) into ISP1238

by transformation with standard DNA from ISP387. Ω40 was thought to be linked to ngr-374 and would serve as a second selectable marker. High MW DNA isolated from ISP1295 was used to transform the nonlysogenic strain ISP1297. Transformants were selected for either Em^r or Thr^+ and scored for Ngr^s on MMS agar. Among the Em^r transformants, 3.3% were Ngr^s Thr^-, only 0.4% were Ngr^s Thr^+, and none were Ngr^r Thr^+ (Table 9). These results suggested the gene order Ω40 - ngr-374 - thrB106. Among the Thr^+ transformants, only 1.7% were Em^r Ngr^s. The failure to recover Em^s Ngr^s transformants may have been due to either the low number of Thr^+ transformants recovered (Table 9) or an effect of ngr-374 on the survivial of these transformants. However, the results of this cross were in agreement with those obtained by Em^r selection, and a single Em^r Ngr^s Thr^+ transformant was isolated and designated ISP1308. In addition to the gene order derived from the data in Table 9, these experiments demonstrated that the presence of ngr-374 in ISP1295 did not affect the genetic activity of DNA isolated from this strain. This observation implied that ngr-374 was not directly involved in the Mod^- phenotype in the Rec^- strains.

ISP1297 and ISP1308 were evaluated for recombination proficiency by transduction with bacteriophage 83A propagated

Table 9. Transformation of ISP1297 with DNA from ISP1295[a]

Frequency[b] of:	Selection for: Em^r	Thr$^+$
Transformation	154	34
Reversion	<10	<10

	Relevant markers Em	Ngr	Thr
Donor:	r	s	+
Recipient:	s	r	-
Analysis of transformants selected for:[c]			
Em^r (1078)			
96.3%	r	r	-
3.3%	r	s	-
0.4%	r	s	+
Thr$^+$ (239)			
98.3%	s	r	+
1.7%	r	s	+

[a] ISP1295 = 8325*r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *lys-115* *trp-103* *ala-126* *tmn-3106* *ilv-126* *pig-131* *hisG15* *nov-142* *ngr-374* Ω[*chr*::Tn*551*]40; ISP1297 = 8325-4*pig-131* *thrB106*.

[b] Expressed as colonies per 10^9 CFU; 3.5×10^8 CFU per ml of transformation suspension and an excess of transforming DNA was used.

[c] Numbers in parentheses are the numbers of transformants examined.

on ISP1465. The results (Table 10) showed that the CP ratios of ISP1297 and ISP1308 were nearly identical to each other. It was evident that ngr-374-carrying strains were Rec$^+$ and that this phenotype was not affected by prophage. However, this conclusion did not rule out the possibility that ngr-374 was one of a number of mutations required for the Rec$^-$ phenotype in RN981. If multiple mutations were needed for the Rec$^-$ phenotype, then the chromosomal position ngr-374 had to be confirmed so that other MNNG sensitive alleles, once identified in RN981, could be differentiated from ngr-374 by chromosomal location. Presently, all of the mapping data for ngr-374 were obtained by the introduction of ngr-374 into ngr$^+$ recipients. To obtain the reciprocal data, ISP1358 was constructed from ISP1238 by re-introducing thrB106 (Table 1). ISP1358 then was transformed with high MW DNA from ISP387 which carries Ω40, with selection for Em^r and Thr$^+$ transformants. Among the Em^r transformants, 61% were Ngr^r Thr$^+$, 10% were Ngr^r Thr$^-$, and only 1.0% were Ngr^s Thr$^+$ (Table 11). Selection for Thr$^+$ gave nearly identical results; 59% of the transformants were Ngr^r Em^r, but only 6.5% were Ngr^r Em^s and only 2.5% were Em^r Ngr^s (Table 11). The high percentage of Em^r Ngr^r Thr$^+$ transformants recovered in this transformation, versus the low percentages of Em^r Ngr^s Thr$^+$

Table 10. Analysis of recombination proficiency of ISP1297 and ISP1308 by transduction with bacteriophage 83A/ISP1465

Strain	Ngr phenotype	Transduction frequency[a]	Correction factor[b]	CP ratio[c]
ISP1297	r	Cad^r = 0.5	-	
		Tmn^r = 0.3	-	0.6
ISP1308	s	Cad^r = 0.3	1.7	
		Tmn^r = 0.2	"	0.7 (116%)

[a] The Cad^r determinant is on pI258, while the Tmn^r determinant is chromosomal. Frequencies are expressed as transductants recovered per 10^6 CFU.

[b] Calculated by dividing the Cad^r transduction frequency for Rec^+ strain (i.e., ISP1297) by the Cad^r transduction frequency for presumptive Rec^- mutant (i.e., ISP1308) and used to normalized the transduction frequency of the presumptive Rec^- mutant to that of the Rec^+ strain.

[c] Ratio of the transduction frequency of the chromosomal markers to the transduction frequency of the plasmid marker (Cad^r). The value in parentheses is the percent of the Rec^+ strain CP ratio exhibited by the presumptive Rec^- mutant.

Table 11. Transformation of ISP1358 with DNA from strain ISP387[a]

Frequency[b] of:	Selection for: Em^r	Thr$^+$
Transformation	3415	995
Reversion	<10	<10

	Relevant markers Em	Ngr	Thr
Donor:	r	r	+
Recipient:	s	s	-
Analysis of transformants selected for:[c]			
Em^r (683)			
61.0%	r	r	+
28.0%	r	s	-
10.0%	r	r	-
1.0%	r	s	+
Thr$^+$ (199)			
59.0%	r	r	+
32.0%	s	s	+
6.5%	s	r	+
2.5%	r	s	+

[a] ISP1358 = 8325r1$^-$ r2$^-$ m31$^+$ m32$^+$ lys-115 trp-103 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374; ISP387 = 8325trp-103 Ω[chr::Tn551]40 ilv-129 pig-131.

[b] Expressed as colonies per 10^9 CFU; 1.0 x 10^8 CFU per ml of transformation suspension and an excess of transforming DNA was used.

[c] Numbers in parentheses are the numbers of transformants examined.

and Em^r Ngr^s Thr^+, suggested a gene order of Ω40 - ngr-374 - thrB106 which was identical to the gene order suggested in earlier transformations.

This transformation was repeated with high MW DNA from ISP803 which carries Ω[chr::Tn551]42, a Tn551 chromosomal insert near Ω40 (Stahl and Pattee, 1983b). Like the previous transformation, selection was for Em^r and Thr^+ transformants, and the results (Table 12) were consistent with the gene order Ω40 - ngr-374 - thrB106. This gene order is summarized in Figure 7, using the data from the transformation of ISP1358 with DNA from ISP803. The map distances in Figure 7 reflect the ambiguity in the positioning of ngr-374 within this linkage group. In all of these transformations, regardless of the selection, ngr-374 was preferentially inherited with the unselected marker. This behavior may be due to the effect of ngr-374 on the survival of the transformants.

To determine if the presences of ngr-374 was responsible for the sensitivity of RN1441 to MNNG (see Table 4), RN1440 and RN1441 were transformed with high MW DNA from ISP387. Selection was for Em^r, and the Ngr phenotype was scored on MNNG agar and MMS agar. Of the Em^r transformants recovered from RN1440, 39% and 21% were Ngr^r when scored on MMS agar and MNNG agar, respectively; similar results were obtained from

Table 12. Transformation of ISP1358 with DNA from strain ISP803[a]

Frequency[b] of:	Selection for: Em^r	Thr$^+$
Transformation	138	79
Reversion	<10	<10

	Relevant markers Em	Ngr	Thr
Donor:	r	r	+
Recipient:	s	s	-
Analysis of transformants selected for:[c]			
Em^r (415)			
80.0%	r	s	-
11.0%	r	r	-
8.0%	r	s	+
1.0%	r	r	+
Thr$^+$ (237)			
89.0%	s	s	+
8.0%	r	r	+
3.0%	s	r	+

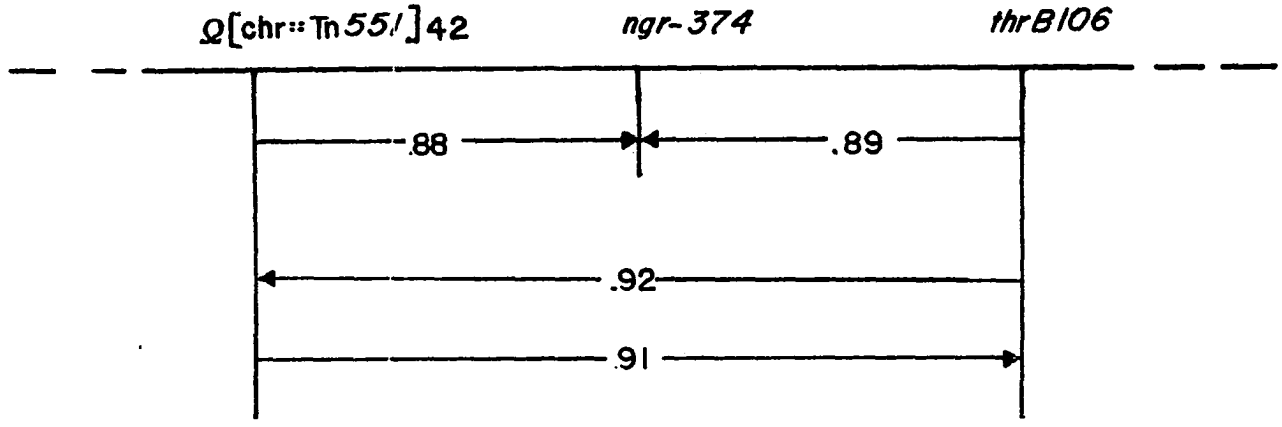
[a] ISP1358 = 8325*r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^+$ *lys-115 trp-103 thrB106 ala-126 tmn-3106 ilv-129 pig-131 hisG15 nov-142 ngr-374*; ISP803 = 8325Ω[*chr*::Tn*551*]*42 pig-131*.

[b] Expressed as colonies per 10^9 CFU; 1.5 x 10^9 CFU per ml of transformation suspension and an excess of transforming DNA was used.

[c] Numbers in parentheses are the numbers of transformants examined.

Figure 7. Linkage of Ω[chr::Tn*551*]42, *ngr-374*, and *thrB106* in *S. aureus* as determined by transformation

Map distances were calculated from the data in Table 12 as 1-estimated co-transformation frequency of the unselected marker. Arrows point towards the unselected markers. Figure is not drawn to scale.


Ω[chr::Tn551]42
ngr-374
thrB106
.88
.89
.92
.91

RN1441. These results showed that the Rec$^+$ revertants of RN981 still carry ngr-374, which explains the sensitivity of RN1441 to MNNG and MMS observed earlier (Table 4). Moreover, the presence of ngr-374 in the Rec$^+$ revertants indicated that this mutation probably does not contribute directly to the Rec$^-$ phenotype in RN981. In a later section, evidence for ngr-374 affecting the AP endonuclease activity in S. aureus will be presented.

Identification of the mit-376 Allele

Another attempt to locate the allele responsible for the Rec$^-$ phenotype of RN981 was made by fusing protoplasts of ISP1171 and RN981. Trp$^+$ Nov^r and Trp$^+$ Tmn^r recombinants were selected from the regenerated cells, and the Rec$^-$ phenotype scored on the basis of UV sensitivity (Uvr^s) by UV irradiating two BHI agar replica plates with 7.2 J/M^2 and 14.4 J/M^2. The results (Table 13) revealed that among the Trp$^+$ Nov$^+$ recombinants, Uvr was coinherited with Nov (94%) and with Tmn (88%). However, except for these two markers, Uvr failed to show significant CIFs with any of the other markers. Similarly, among the Trp$^+$ Tmn^r recombinants, Uvr showed high a CIF with Tmn (94%), but a CIF of only 30% with Nov, and as in the Trp$^+$ Tmn^r selection, Uvr failed to show significant CIFs

Table 13. Coinheritance frequencies for an RN981 x ISP1171 protoplast fusion[a]

Coinheritance frequencies (%) of Trp^+ Nov^r recombinants[b]								
	Ilv	Ura	Nov	Thr	Trp	Tyr	Tmn	Uvr
Ilv	100	79	27	69	73	82	33	24
Ura	79	100	16	79	84	82	21	16
Nov	27	16	100	10	0	19	92	94
Thr	69	79	10	100	90	76	15	15
Trp	73	84	0	90	100	81	8	6
Tyr	82	82	19	76	81	100	23	16
Tmn	33	21	92	15	8	23	100	88
Uvr	24	16	94	15	6	16	88	100

Coinheritance frequencies (%) for Trp^+ Tmn^r recombinants[b]								
	Ilv	Ura	Nov	Thr	Trp	Tyr	Tmn	Uvr
Ilv	100	84	80	88	79	81	21	18
Ura	19	100	74	84	81	83	19	18
Nov	34	74	100	81	66	69	34	30
Thr	16	84	81	100	84	84	16	14
Trp	79	81	66	84	100	90	0	5
Tyr	81	83	69	84	90	100	10	11
Tmn	20	19	34	16	0	10	100	94
Uvr	18	18	30	14	6	11	94	100

[a] RN981 = Thr^+ Uvr^s Trp^+ Tyr^+ Tmn^s Ilv^+ Ura^+ Nov^s; ISP1171 = Thr^- Uvr^r Trp^- Tyr^- Tmn^r Ilv^- Ura^- Nov^r $\phi 11^s$ $\phi 12^s$.

[b] The numbers represent the percentages of the total numbers of recombinants with either of the parental phenotypes for the indicated pairs of markers (Stahl and Pattee, 1983a).

with any of the other markers. These results suggested that two Uvr^S alleles might be present in RN981, one near nov-142 and the other near tmn-3106 (Fig. 6, segments 5 and 10, respectively), and together they might be responsible for the Rec^- phenotype of RN981.

The mapping of the Uvr alleles was attempted by transforming ISP1237 with high MW DNA from RN981. Since RN981 carried neither nov-142 or tmn-3106, His^+ and Ilv^+ transformants were selected. Because of the lysogenic nature of ISP1237 it was necessary to score the Uvr alleles on MNNG agar. Goering (1979) reported that Rec^- S. aureus were both Uvr^S and $MNNG^S$, predicting that if one of the Uvr^S alleles was indeed recA1, then transformants carrying this allele should be $MNNG^S$. Also, because of the chromosomal location of ngr-374, there was little concern that scoring the His^+ and Ilv^+ transformants on MNNG agar would result in the re-isolation of ngr-374. For clarity, the sensitivity of these transformants to MNNG was referred to as Mng^S.

Among the Ilv^+ and His^- transformants, Mng^S appeared only among the His^+ transformants. Of 138 His^+ transformants examined, 65 were Mng^S and six of these were isolated. These six isolates were then examined by disk assay for sensitivity to EMS, MMS, and MitC (Table 14). The Rec^+ parent, ISP1237,

Table 14. Disk assay of ISP1237 Mng^s transformants

Strain	Mng phenotype	Zones of inhibition (mm)[a]		
		EMS[b]	MMS	MitC
ISP1237	r	0	44	32
RN981	s	25	60	30
Clone:[c]				
1	s	26	60	38
2	s	0	45	36
4	s	26	60	40
6	s	29	62	40
7	s	32	68	40
9	s	28	64	45

[a] Values are the diameters of the zones of inhibition from a single experiment.

[b] EMS = ethyl methanesulfonate; MMS = methyl methanesulfonate; MitC = mitomycin C.

[c] His^+ Mng^s transformants isolated from transformation of ISP1237 with RN981 DNA.

was slightly sensitive to MMS and MitC, but because of the resistance of this strain to EMS, these sensitivities were attributed to prophage induction (EMS does not readily induce prophage; Kondo et al., 1970). Five of the six isolates showed sensitivities equal to or greater than RN981 to all the agents tested. The similarities in sensitivity to these agents between these isolates and RN981 suggested that the Mng^S allele, which cotransformed with His^+, was probably one of the Uvr^S allele identified by protoplast fusion and quite possibly responsible for the Rec^- phenotype of RN981. Isolates 9 and 7 (Table 14) were retained for further study as ISP1313 and ISP1314, respectively.

When assayed for recombination proficiency, the Mng^S mutant, ISP1314, had a CP ratio of 0.05, which was 36% of the CP ratio for the same marker in Rec^+ ISP1237 (Table 15). This reduction in recombination suggested that the Mng^S allele did impair genetic recombination, and that the residual recombination in ISP1314 was due to either complementation of this allele by prophage or the absence of another mutation present in recA1 strains and required to completely inactivate recombination in S. aureus.

The role of the Mng^S allele in the Rec^- phenotype in RN981 was confirmed by transforming the Rec^+ revertant RN1441 with

Table 15. Analysis of recombination proficiency in ISP1237 and ISP1314 by transduction with bacteriophage 83A/ISP1465

Strain	Mng phenotype	Transduction frequency[a]	Correction factor[c]	CP ratio[d]
ISP1237	r	Cad^r = 3.5	-	
		Trp^+ = 4.5	-	1.4
ISP1314	s	Cad^r = 0.2	17.5	
		Trp^+ = 0.1	"	0.5 (36%)

[a] The Cad^r determinant is on pI258, while the Trp^+ determinants is chromosomal. Frequencies are expressed as transductants recovered per 10^6 CFU.

[b] Calculated by dividing the Cad^r transduction frequency for Rec^+ strain (i.e., ISP1237) by the Cad^r transduction frequency for presumptive Rec^- mutant (i.e., ISP1314) and used to normalized the transduction frequencies of the presumptive Rec^- mutant to that of the Rec^+ strain.

[c] Ratio of the transduction frequency of the chromosomal markers to the transduction frequency of the plasmid marker (Cad^r). The value in parentheses is the percent of the Rec^+ strain CP ratio exhibited by the presumptive Rec^- mutant.

standard DNA from ISP1314 and selecting for His^+. Since RN1441 was originally isolated by screening RN981 for Mit^r revertants (Wyman et al., 1974), the conversion of RN1441 back to Rec^- by the introduction of the Mng^s allele was scored on MitC agar. When RN1441 was transformed, only 82 His^+ transformants were recovered. However, 12 of these His^+ transformants were Mit^s and Rec^- by transduction analysis (data not shown), thus indicating that the Mng^s allele was involved in the Rec^- phenotype in S. aureus. This allele, responsible for Mng^s, Mit^s, and Rec^- in RN1441, and for the reduction in recombination in ISP1314, was designated mit-376. This designation was in preference to recA1 since it still had not been established that this allele was responsible for all of the phenotypes attributed to RN981.

The effect on recombination of having both ngr-374 and mit-376 in a nonlysogenic background was examined by transforming ISP1402 and ISP1404 with standard DNA from ISP1314. The latter strain is isogenic to ISP1402 except that it carries the ngr-374 mutation. Selection was for His^+ transformants, and mit-376 was scored for on MitC agar. Transformation of ISP1402 resulted in the recovery 296 His^+ transformants of which 19% were Mit^s and 8% were also Nov^s, but only 1.0% were Mit^r Nov^s (Table 16). These results

Table 16. Transformation of ISP1402 with DNA from strain ISP1314[a]

	Selection for:
Frequency[b] of:	His^+
Transformation	104
Reversion	<10

	Relevant markers		
	Nov	Mit	His
Donor:	s	s	+
Recipient:	r	r	-
Analysis of 296 His^+ transformants			
80.0%	r	r	+
11.0%	r	s	+
8.0%	s	s	+
1.0%	s	r	+

[a] ISP1402 = 8325-4 *purA102 nov-142 hisG15 pig-131 thrB106*; ISP1314 = 8325 *r1⁻ r2⁻ m31⁺ m32⁻ tyrB282::Tn551 ermB321 trp-159::Tn551 ermB317 thrB106 ala-126 tmn-3106 ilv-126 nov-142 mit-376*.

[b] Expressed as colonies per 10^9 CFU; 1.5 x 10^9 CFU/ml per ml of the transformation suspension and excess of transforming DNA were used.

suggested the gene order nov-142 - mit-376 - hisG15. A single Mit^s Nov^r clone was purified and retained as ISP1403. Similar results were obtained from the transformation of ISP1404. In this transformation, Mit^s cotransformed with His^+ 31% of the time, with 14% being Mit^s Nov^s while only 7% were Mit^r Nov^s (Table 17) suggesting that the gene order was identical to that predicted previously. A single Mit^s Nov^r clone from this transformation was isolated and designated ISP1405.

The recombination proficiency of ISP1403, ISP1405, and a third mit-376-carrying strain, ISP1464, was evaluated by transduction. ISP1464 was isolated by transforming ISP1407, a ϕ12 lysogen, with standard DNA from ISP1314, selecting for His^+, and scoring for mit-376 on MNNG agar. All three of the mit-376-carrying strains had CP ratios which were only 6.0 to 11.0 % of their respective parental strains (Table 18). These results indicated that mit-376 caused an approximate 90% reduction in recombination in the nonlysogens and the ϕ12 lysogen. Furthermore, the presence or absence of ngr-374 had no effect on the level of reduction in recombination (Table 18).

The mit-376-carrying strains were also examined for the effect of this mutation on DNA modification (Mod). These experiments were performed in spite of the ability of DNA from

Table 17. Transformation of ISP1404 with DNA from strain ISP1314[a]

	Selection for:
Frequency[b] of:	His^+
Transformation	98
Reversion	<10

	Relevant markers		
	Nov	Mit	His
Donor:	s	s	+
Recipient:	r	r	-
Analysis of 296 His^+ transformants			
62.0%	r	r	+
17.0%	r	s	+
14.0%	s	s	+
7.0%	s	r	+

[a] ISP1404 = 8325-4 *purA102 nov-142 hisG15 pig-131 thrB106 ngr-374* Ω[chr::Tn*551*]40; ISP1314 = 8325 *r1⁻ r2⁻ m31⁺ m32⁻ tyrB282*::Tn*551 ermB321 trp-159*::Tn*551 ermB317 thrB106 ala-126 tmn-3106 ilv-126 nov-146 mit-376*

[b] Expressed as colonies per 10^9 CFU; 9.9 x 10^8 CFU/ml per ml of the transformation suspension and excess of transforming DNA were used

Table 18. Analysis of recombination proficiency in strains of S. aureus carrying mit-376 by transduction with bacteriophage 83A/ISP1465

Strain	Pertinent genotypes	Transduction frequency[a]	Correction factor[b]	CP ratio[c]
ISP1402		Cad^r = 0.07	-	
		Tmn^r = 0.2	-	2.9
ISP1403	mit-376	Cad^r = 0.4	0.18	
		Tmn^r = 0.1	"	0.3 (10%)
ISP1404	ngr-374	Cad^r = 0.4	-	
		Tmn^r = 0.3	-	0.8
ISP1405	mit-376	Cad^r = 0.4	1.0	
	ngr-374	Tmn^r = 0.02	"	0.05 (6%)

[a] The Cad^r determinant is on pI258, while the Tmn^r determinant is chromosomal. Frequencies are expressed as transductants recovered per 10^6 CFU.

[b] Calculated by dividing the Cad^r transduction frequency for Rec^+ strain (e.g., ISP1402) by the Cad^r transduction frequency for presumptive Rec^- mutant (e.g., ISP1403) and used to normalized the transduction frequencies of the presumptive Rec^- mutant to that of the Rec^+ strain.

[c] Ratio of the transduction frequency of the chromosomal markers to the transduction frequency of the plasmid marker (Cad^r). The values in parentheses are the percent of the Rec^+ strain CP ratio exhibited by the presumptive Rec^- mutant.

Table 18. (continued)

Strain	Pertinent genotypes	Transduction frequency	Correction factor	CP ratio
ISP1407	(ϕ12)[d]	Cad^r = 0.08	-	
		Tmn^r = 0.1	-	1.3
ISP1464	mit-376	Cad^r = 0.4	0.1	
	(ϕ12)	Tmn^r = 0.06	"	0.15 (12%)

[d] Lysogenic for bacteriophage ϕ12.

ISP1314 to transform restriction-proficient Rec$^+$ recipients, because it was unknown what effect loss of DNA modification might have on the genetic activity of DNA from a mit-376-carrying restrictionless strain (e.g., ISP1314). Bacteriophage 80α lysates propagated on ISP1403 and ISP1405 exhibited the same efficiency of plating on ISP8 and the propagating strain. Apparently, the Mod$^-$ phenotype was not directly associated with mit-376, but, like ngr-374, probably caused by a mutation not associated with genetic recombination.

The occurrence of post-UV irradiation DNA degradation (i.e., reckless DNA repair; Goering, 1979) in the mit-376-carrying strains was determined by DNA degradation assays (Table 19). RN981 (recA1) showed UV-induced DNA degradation by releasing 31% of its labelled DNA after UV irradiation. In contrast, RN1441 (mit-6) released approximately 2.5-fold less label after UV irradiation than RN981, and neither strain showed any evidence of spontaneous DNA degradation (i.e., percent label released from non-irradiated cells). ISP1403 (mit-376) and ISP1405 (ngr-374 mit-376) showed levels of DNA degradation after UV irradiation which equalled or approached that seen in RN981. Spontaneous DNA degradation was exhibited by only ISP1403 and ISP1405;

Table 19. Post-UV irradiation DNA degradation in *mit-376*-carrying strains of *S. aureus*

Strains	Relavent genotype	Percent of the total label released		
		Spontaneous[a]	UV irradiated[b]	Net[c]
RN981	*recA1*	5.8	36.7	31.0
RN1441	*mit-6*	3.1	15.3	12.3
ISP1402		1.9	6.4	4.6
ISP1403	*mit-376*	13.2	39.0	31.6
ISP1404	*ngr-374*	3.0	17.9	14.7
ISP1405	*ngr-374* *mit-376*	11.9	34.6	22.6

[a] Percent of total label released from nonirradiated cells.

[b] Percent of total label released from cells after exposure to 6 J/M^2.

[c] Percent of label released after UV irradiation minus percent of label released spontaneously.

however, the significance of this activity remains unknown. In addition, ISP1404, which carries ngr-374, but is Rec$^+$, showed a significantly higher level of DNA degradation after UV irradiation than ISP1402 which, except for ngr-374, is isogenic to ISP1404. This observation suggested that the ngr-374 mutation may impair the repair of UV-generated lesions. Based on this and earlier data it was apparent that mit-376 accounted for most of the phenotypes associated with recA1 mutants with the exception of DNA modification.

With the phenotypes associated with mit-376 determined, confirmation of the position of mit-376 within the linkage group purA102 - nov-142 - hisG15 was sought by transformation. ISP1359 was transformed with standard DNA from RN981 with selection for Pur$^+$ and His$^+$ transformants. Among the 691 Pur$^+$ transformants examined, 43% were Mng^S (mit-376). Of these, the most significant classes were Nov^S Mng^S His$^-$ and Nov^S Mng^S His$^+$, occurring in 34% and 8% of the transformants, respectively (Table 20). The remaining Mng^S transformants were Nov^r Mng^S His$^+$ and Nov^r Mng^S His$^-$, each of which occurred in less than 1% of the transformants. Likewise, Nov^r Mng^r His$^-$ and Nov^S Mng^r His$^+$ transformants were rare, occurring in only 2.2% and 0.1% of the transformants examined, repectively. From these data, the gene order purA102 - nov-142 - mit-376 -

Table 20. Transformation of ISP1359 with DNA from strain RN981[a]

Frequency[b] of:	Selection for: Pur$^+$	His$^+$
Transformation	1114	1198
Reversion	<10	<10

	Relevant markers: Pur	Nov	Mng[c]	His
Donor:	+	s	s	+
Recipient:	-	r	r	-
Analysis of transformants selected for:[d]				
Pur$^+$ (691)				
51.0%	+	r	r	-
34.0%	+	s	s	-
8.3%	+	s	s	+
3.0%	+	r	r	+
2.2%	+	s	r	-
0.4%	+	r	s	+
0.3%	+	r	s	-
0.1%	+	s	r	+

[a] ISP1359 = 8325*r1*$^-$ *r2*$^-$ *m31*$^+$ *m32*$^-$ *tyrB282*::Tn*551* *trp-103* *thrB106* *ala-126* *tmn-3106* *ilv-126* *nov-142* *purA102* *hisG15*; RN981 = 8325-4*his-7* *recA1* *pig-131*.

[b] Expressed as colonies per 10^9 CFU; 3.1×10^8 CFU per ml of transformation suspension and an excess of transforming DNA was used.

[c] *mit-376* scored on MNNG (Mng^s).

[d] Numbers within the parentheses are the numbers of transformants examined.

Table 20. (Continued)

	Relevant markers			
	Pur	Nov	Mng	His
Donor:	+	s	s	+
Recipient:	-	r	r	-
Analysis of transformants selected for:				
His^+ (599)				
40.0%	-	s	s	+
37.0%	-	r	r	+
20.0%	+	s	s	+
1.7%	-	r	s	+
1.3%	+	r	r	+

hisG15 was proposed. When selected for His^+ transformants, 61.7% of the 599 transformants examined had coinherited Mng^S. Consistent with the results obtained from the Pur^+ selection, the major class observed was Pur^- Nov^S Mng^S (40%), followed by Pur^+ Nov^S Mng^S (20%). The remaining Mng^S transformants (1.7%) belonged to the Pur^- Nov^r Mng^S class. These results were supportive of the gene order proposed from the data obtained with Pur^+ selection. This gene order with estimated map distances is summarized in Figure 8. Figure 9 shows the positions of ngr-374 and mit-376 on the S. aureus chromosome.

Characterization of the ngr-374 Allele

The genetic analysis of RN981 has shown that this strain contains at least two mutations which can affect the sensitivity of S. aureus to DNA alkylating agents. One of these, mit-376, has been shown to impair recombination functions; however, the nature of the of the other, ngr-374, was uncertain. To examine this situation further, the sensitivity of the ngr-374-carrying strain ISP1238 to MNNG and MMS was examined by an inactivation assay (Fig. 10). When ISP1238 was treated with 100 µg/ml of MNNG it was a 20-fold more sensitive to MNNG than the isogenic Ngr^r strain ISP926 (Fig. 10A). To determine if this sensitivity was unique to

Figure 8. Linkage of purA102, nov-142, mit-376, and hisG15 in S. aureus as determined by transformation

Map distances were calculated from the data in Table 20 as 1 - estimated co-transformation frequency of the unselected marker. Arrows point towards the unselected markers. Figure is not drawn to scale.

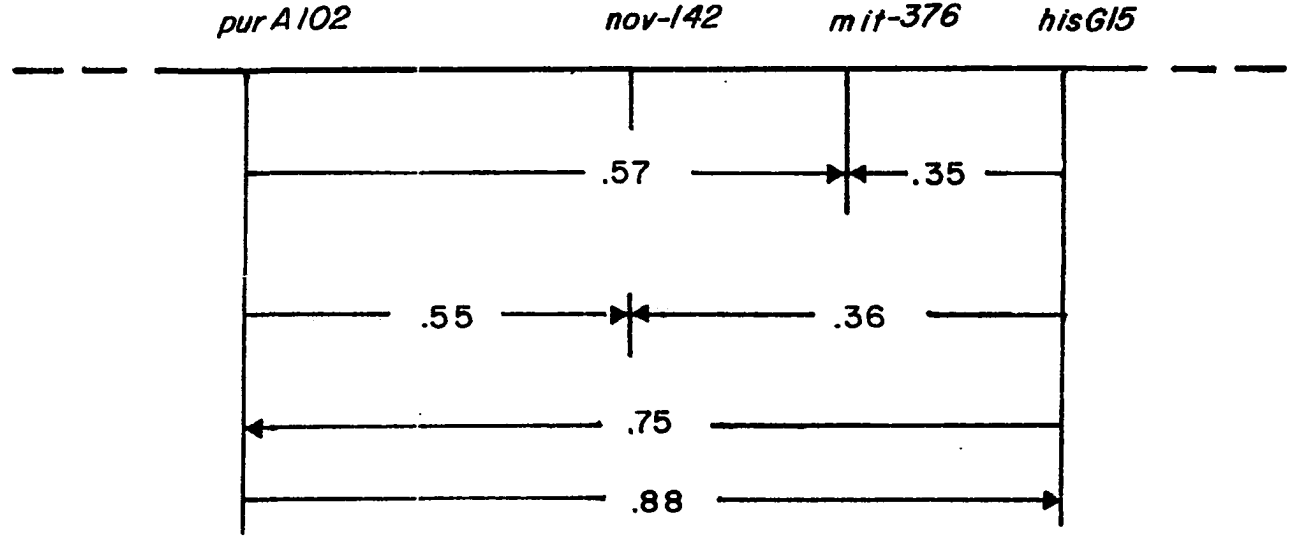

purA102
nov-142
mit-376
hisG15
.57
.35
.55
.36
.75
.88

Figure 9. Chromosomal map of S. aureus NCTC 8325, showing the position of mit and ngr

Figure is modified form of Fig. 6 and shows the position of ngr (segment 16) and mit (segment 10). Markers within parentheses have not been orientated relative to their adjacent markers. Broken lines indicate regions where linkage has not been established (segments 8, 11, and 13). Sites of Tn551 insertion are designated by Ω followed by an isolation number (except Ω420 and Ω500 which are insertions of the plasmid pI258).

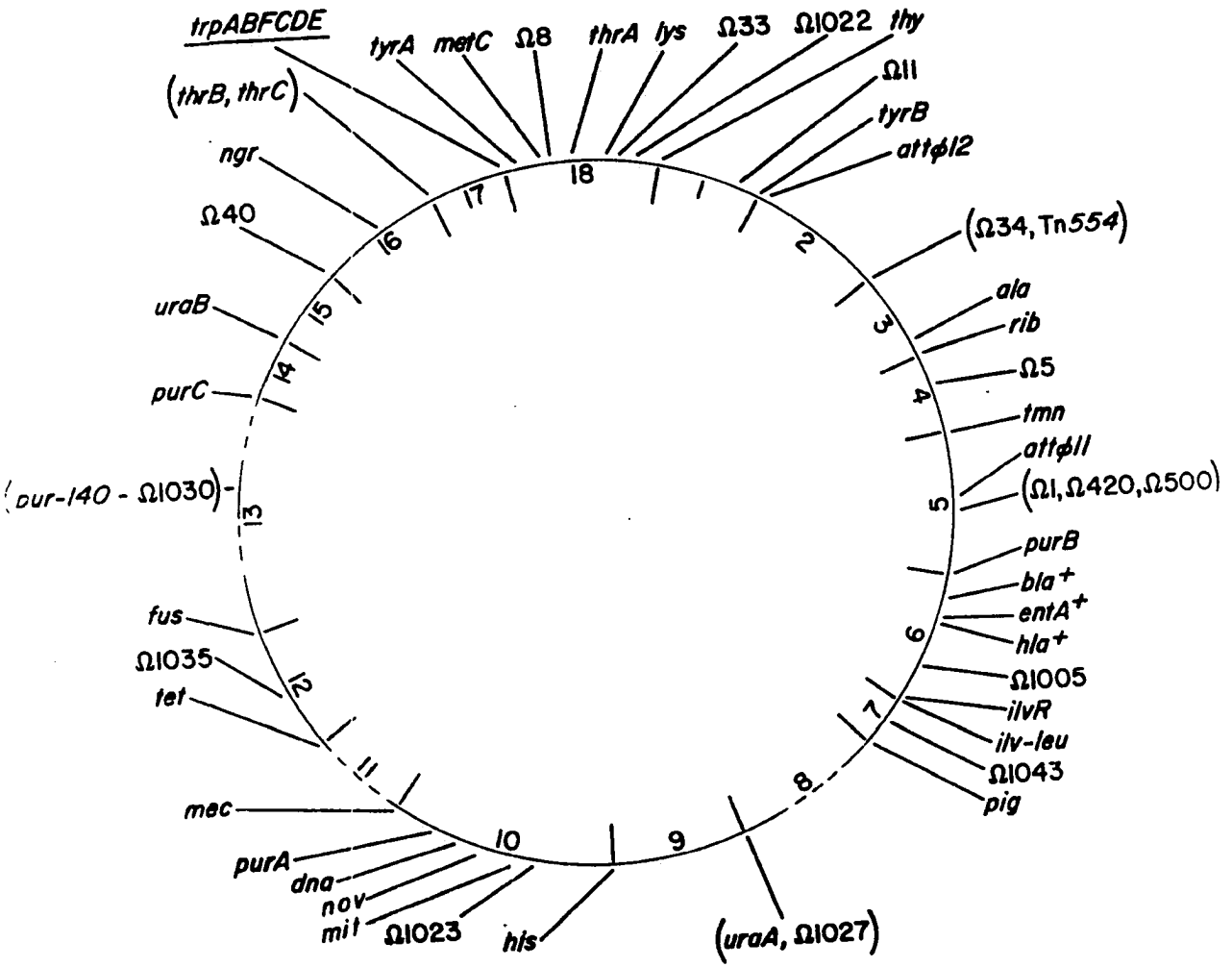
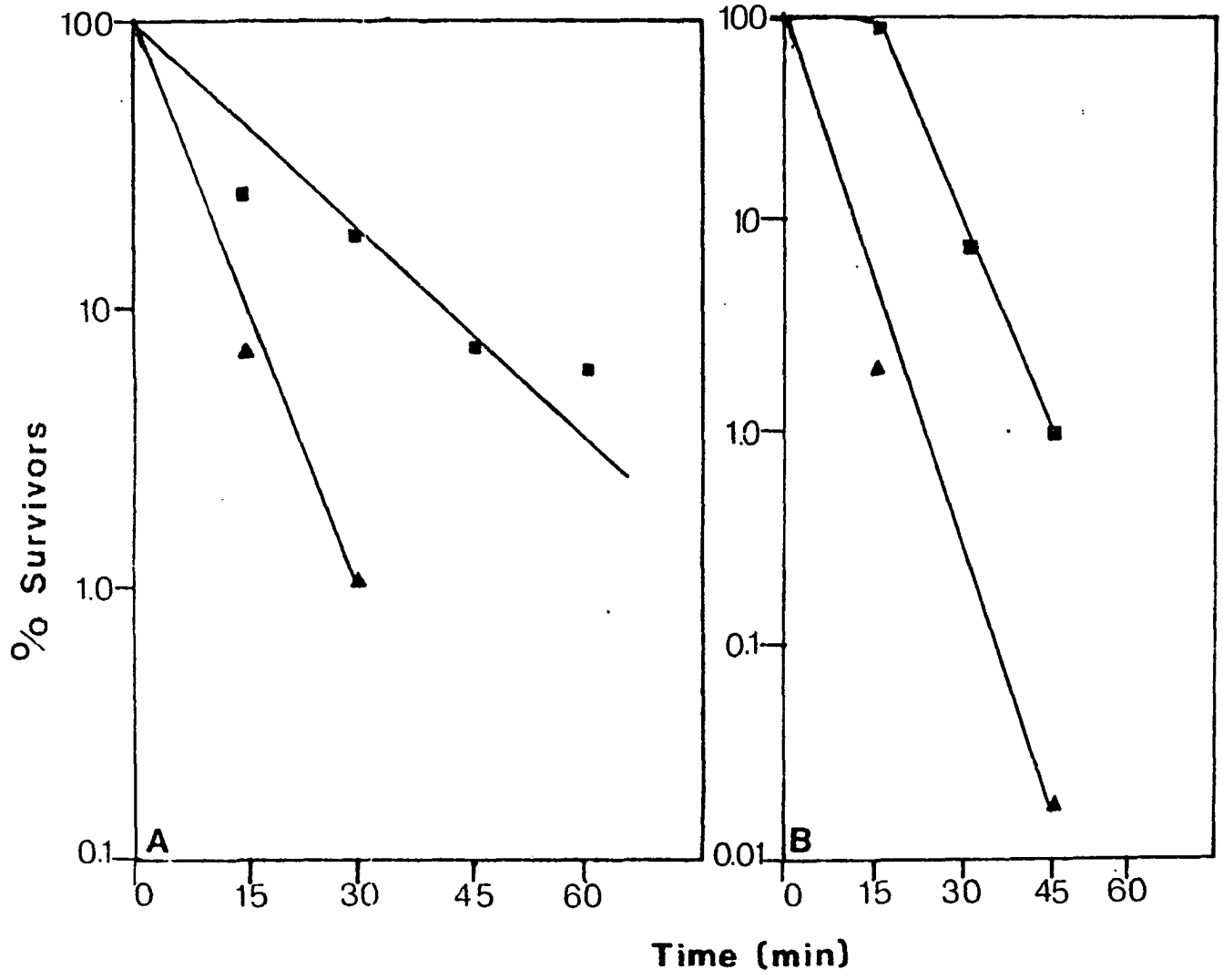

trpABFCDE
tyrA
metC
Ω8
thrA
lys
Ω33
Ω1022
thy
Ω11
tyrB
attφ12
(Ω34, Tn554)
ala
rib
Ω5
tmn
attφ11
(Ω1, Ω420, Ω500)
purB
bla+
entA+
hla+
Ω1005
ilvR
ilv-leu
Ω1043
pig
(uraA, Ω1027)
his
Ω1023
mit
nov
dna
purA
mec
tet
Ω1035
fus
(pur-140 - Ω1030)
purC
uraB
Ω40
ngr
(thrB, thrC)
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18

Figure 10. Inactivation assays of ISP926 and ISP1238 with MNNG and MMS

A. The response of ISP1238 (*ngr*$^{+}$; ■) and ISP926 (*ngr-374*; ▲) to 100 µg/ml MNNG. B. Response of ISP926 and ISP1238 to 5 mM MMS; symbols are the same as in 10A.


100
10
1.0
0.1
% Survivors
A
0
15
30
45
60
100
10
1.0
0.1
0.01
B
0
15
30
45
60
Time (min)

MNNG, ISP1238 was treated with 5 mM MMS in a similar assay; MMS treatment produces a different spectrum of DNA products from MNNG treatment (Strauss et al., 1974). As with MNNG treatment, ISP1238 was approximately 20-fold more sensitive to MMS than ISP926 (Fig. 10B) which suggested that ngr-374 impaired the repair of alkylated DNA. Based on the E. coli models for repair of alkylated DNA, it was thought that ngr-374 might be causing a deficiency in either a DNA-N-glycosylase specific for a product common MMS and MNNG treatments or in an AP endonuclease (Linn, 1978).

Disk assays were used to determine the spectrum of ngr-374 sensitivity. The results shown in Table 21 indicated that ngr-374-carrying strains have increased sensitivity to the DNA alkylating agents EMS and MMS, but not to to MitC and NQO. Even the lysogenic mutant, ISP1238, which suffered from prophage induction on MMS, MitC, and NQO, was visibly more sensitive to the DNA alkylating agents. These results supported the belief that ngr-374 was impairing a repair system in S. aureus which was specific for alkylated DNA.

Host cell reactivation (HCR) experiments were used to test for a deficiency in a DNA-N-glycosylase specific for products common to MNNG, MMS, or EMS. Bacteriophage 80α was treated with 50 mM MMS and titered on ISP926, ISP1238, and ISP1185;

Table 21. Disk assay of S. aureus sensitivity to various DNA modifying agents

Strain	Ngr phenotype	Prophage content	Zones of inhibition (mm)[a]			
			EMS[c]	MMS	MitC	NQO
ISP8	r	none	0	20	20	0
ISP926	r	ϕ11,ϕ12,ϕ13	0	54	40	25
ISP1185	s	ϕ11	39	62	35	30
ISP1238	s	ϕ11,ϕ12,ϕ13	23	62	40	26
ISP1297	r	none	0	20	26	0
ISP1308	s	none	23	54	28	0

[a] Values are the diameters of the zones of inhibition from a single experiment.

[b] EMS = ethyl methanesulfonate; MMS = methyl methanesulfonate; MitC = mitomycin C; NQO = 4-nitro-quinoline-1-oxide.

the latter strain was Rec⁻. As shown in Figure 11, all of the strains have the same HCR activity for MMS-treated bacteriophage 80α. If ngr-374 was a mutation in a DNA-N-glycosylase specific to a product common to MMS, EMS, and MNNG treatments, then a dramatic difference would have been expected between the HCR activities of ISP1238 and ISP926 for MMS-treated bacteriophage (Karren et al., 1980; Stuy and Bagci, 1983). However, the similarity in the HCR activities of these strains strongly suggest that ngr-374 does not affect such a DNA-N-glycosylase.

Since ngr-374 seemed not to be a mutation affecting a S. aureus DNA-N-glycosylase, ISP1238 was examined for the loss of AP endonuclease activity. Da Roza et al. (1977) reported that the xthA mutants of E. coli, deficient in the AP endonuclease ExoIII, were sensitive to nitrous acid treatment. It was assumed that this sensitivity was caused by the inability of these mutants to repair AP sites formed through the removal of the deaminated bases by DNA-N-glycosylases (Da Roza et al., 1977). When treated with 50 mM nitrous acid, the ngr-374-carrying ISP1238 was twice as sensitive as ISP926 to nitrous acid, and responded in a linear fashion to the treatment (Fig. 12). ISP926, on the other hand, showed a curvilinear response to the treatment. The linear response of

Figure 11. Host cell reactivation of S. aureus bacteriophage 80α

Plots show the survivial of plaque-forming activity of bacteriophage 80α treated with MMS. Lysates of bacteriophage 80α containing 10^{11} PFU/ml were incubated at 37°C for the time intervals indicated in TSB containing 50 mM MMS. Samples were removed, serially diluted ten-fold in TSB and titered on ISP926 (ngr$^+$; ■), ISP1185 (recA1; ●), and ISP1238 (ngr-374; ▲).

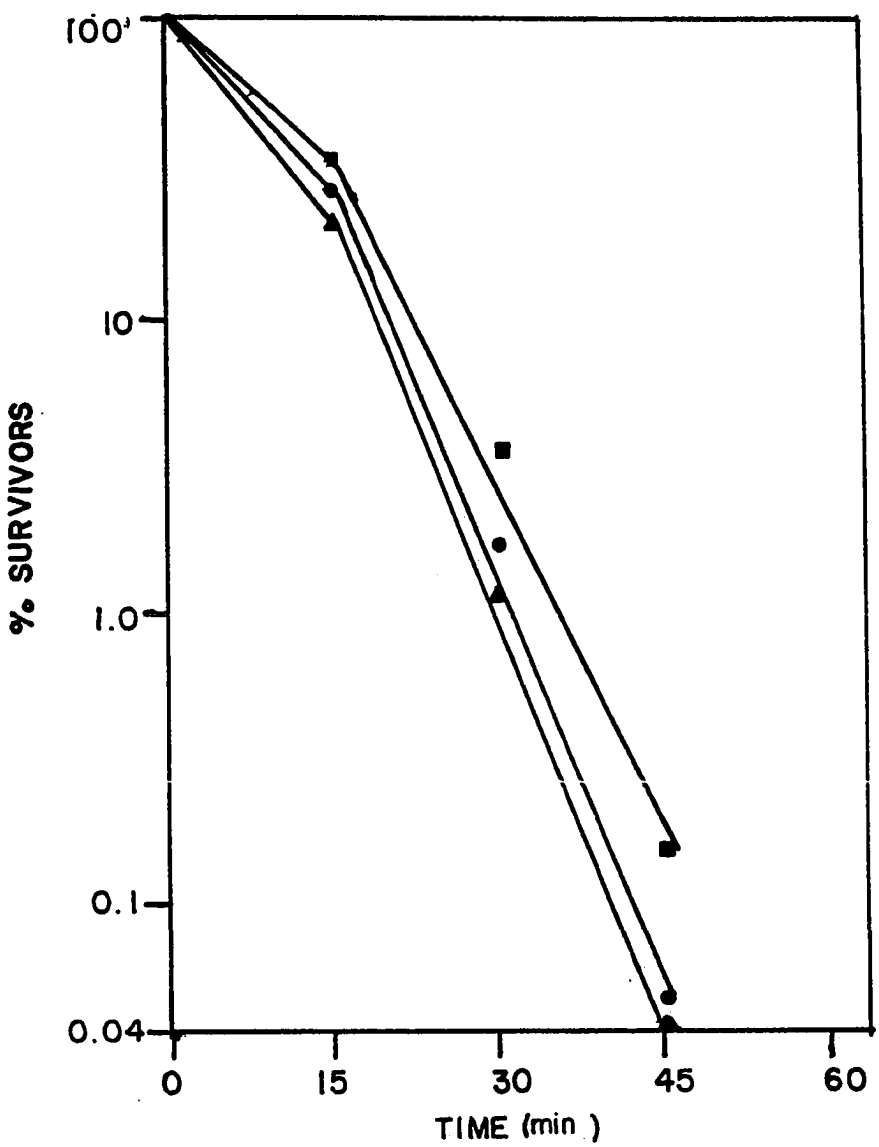
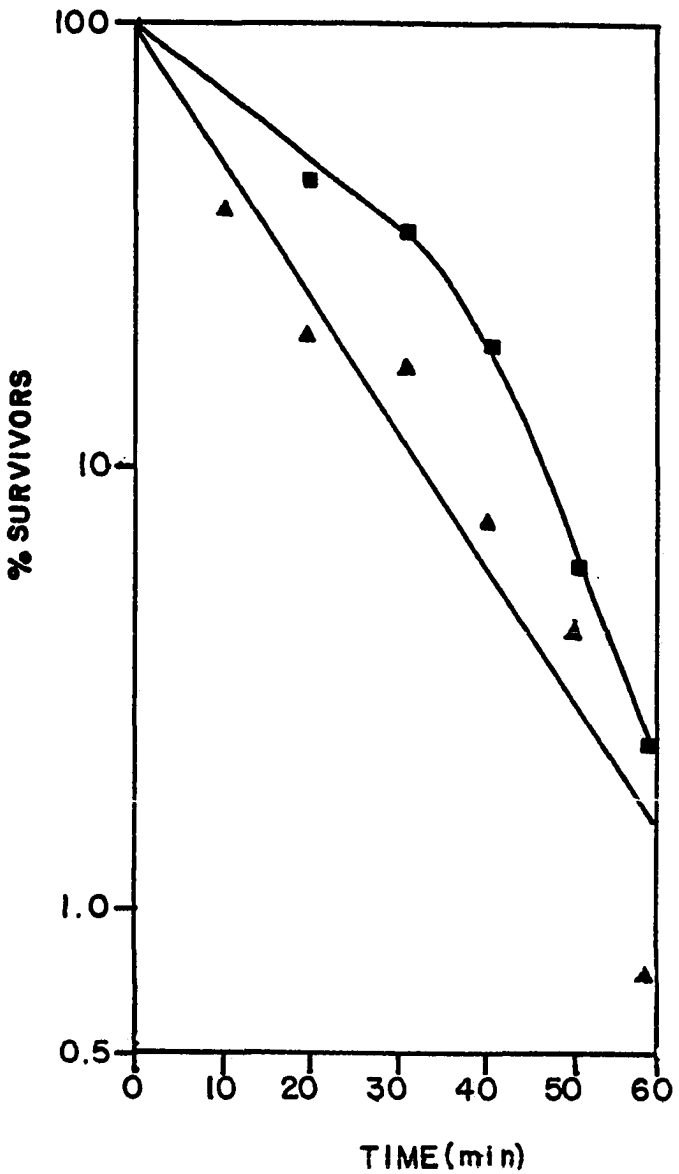

100
10
1.0
0.1
0.04
% SURVIVORS
0
15
30
45
60
TIME (min)

Figure 12. Nitrous acid inactivation of ISP926 and ISP1238
ISP926 (*ngr*$^{+}$; ■) and ISP1238 (*ngr-374*; ▲) were exposed to 50 mM nitrous acid.


100
10
1.0
0.5
% SURVIVORS
0
10
20
30
40
50
60
TIME(min)

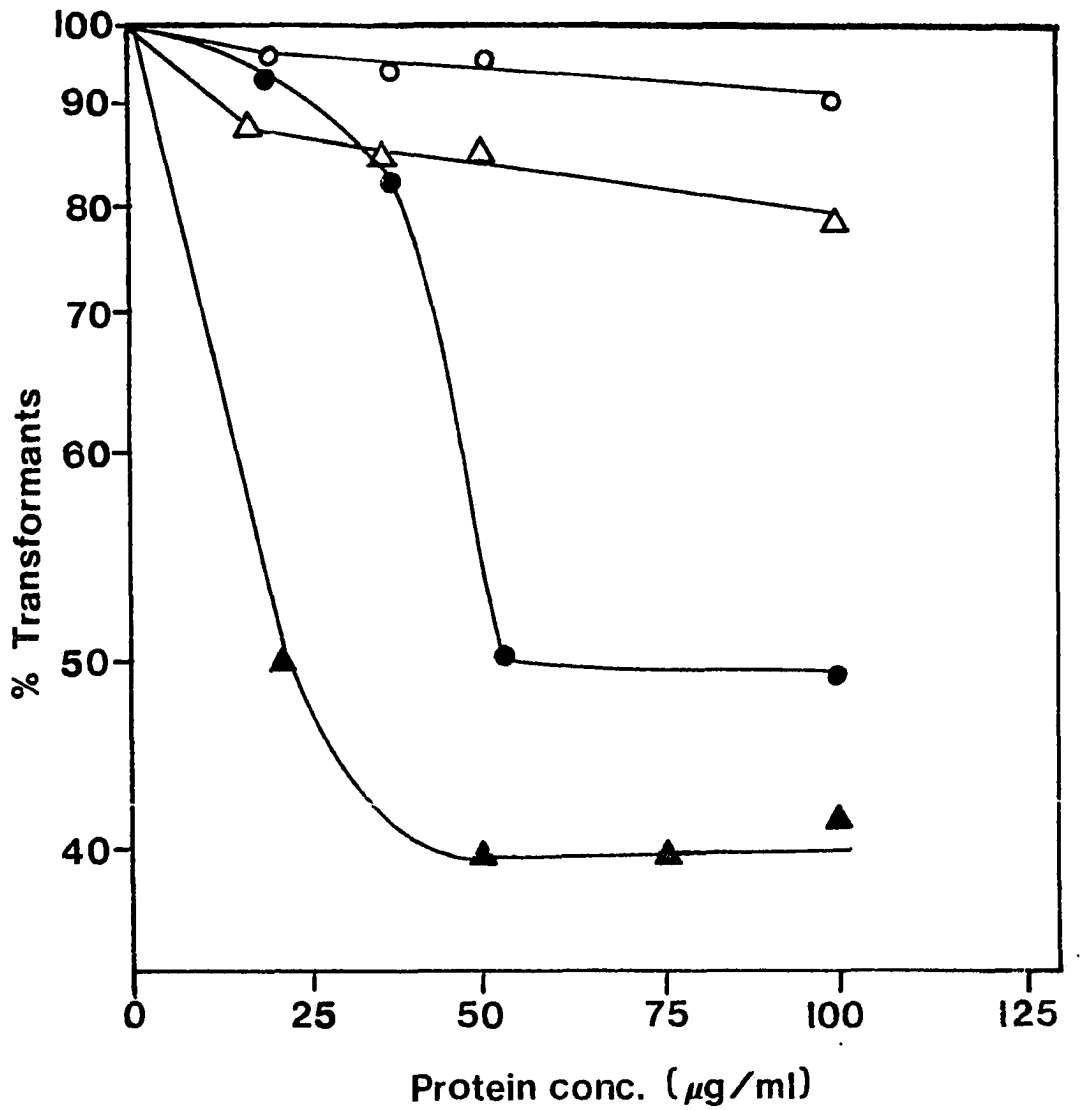
ISP1238 suggested that every lesion generated by nitrous acid treatment was lethal, while the curvilinear response of ISP926 suggested that repair of the lesions generated by nitrous acid was occurring at the lower dosages. The data shown in Figure 12 were analyzed by linear regression analysis; correlation coefficients of -0.95 and -0.80 were calculated for ISP1238 and ISP926, respectively. These coefficients were supportive of the plots as they were drawn in Figure 12. Based on these data and the phenotypes already described, it was believed that ngr-374 was a mutation affecting the AP endonuclease activity in S. aureus.

Cell-free extracts from ngr-374-carrying ISP1238 and ISP926 were assayed for AP endonuclease activity using the B. subtilis bioassay described by Ljundquist et al. (1976). In this bioassay, DNA from strain A-62 was depurinated and used to transform strain SB68. When depurinated DNA is incubated in the presence of an AP endonuclease, nicks are introduced into the DNA at the sites of depurination (i.e., AP sites) which causes a reduction in the transformation activity of the DNA (Strauss and Wahl, 1964; Ljundquist et al., 1976). When the AP endonuclease activity in cell-free extracts from ISP1238 and ISP926 were examined and the percent of SB68 Trp^+ transformants were used as an indicator of the transformation

activity of the depurinated DNA, a rapid drop in the transformation activity of depurinated DNA was evident at fairly low concentrations of protein in ISP926 cell-free extracts (Fig. 13). However, cell-free extracts from ISP1238 showed only a gradual reduction in the transformation activity of the depurinated DNA at protein concentrations less than 50 µg/ml, but as the protein concentration increased beyond 50 µg/ml the transformation activity dropped precipitously to a level nearly equal to ISP926 (Fig. 13). These results indicated that ISP1238 cell-free extracts have a lower level of AP endonuclease activity and the recovery of AP endonuclease activity at the higher concentrations of protein might be attributed to either a leakiness in the ngr-374 mutation or complementation by other S. aureus nucleases having limited AP endonuclease activity. When cell-free extracts were incubated with nondepurinated DNA, a decrease of only 10% and 20% in the transformation activity with ISP1238 and ISP926 cell-free extracts, respectively, were observed. These levels remained unchanged as the protein concentration increased. The results of this bioassay were analogous to those reported for xthA mutants of E. coli (Ljundquist et al., 1976) and support the conclusion that the presence of ngr-374 caused a reduction in the S. aureus AP endonuclease activity.

Figure 13. Bioassay of AP endonuclease activity in ISP926 and ISP1238

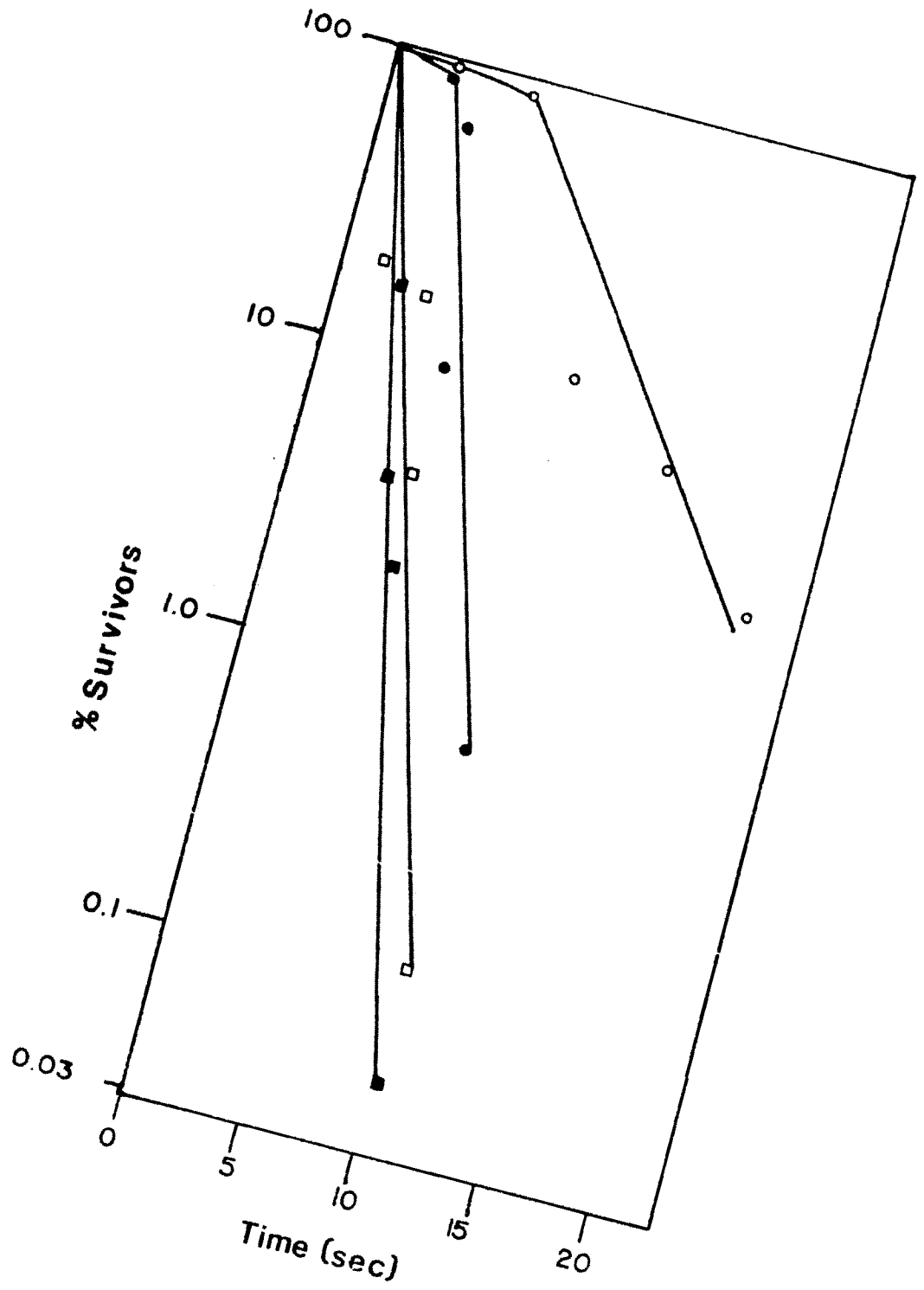
The AP endonuclease activity was assayed by treating depurinated A-62 DNA with different concentrations of cell-free extract from ISP926 (▲) and ISP1238 (●) and determining the effect of these treatments on the transformation activity of the DNA. The transformation activities are expressed as percent of Trp^+ transformants recovered, with 100% transformants equal to the number of Trp^+ transformants recovered from transformations with depurinated A-62 DNA not treated with cell-free extract. Between 10^3 and 10^4 Trp^+ transformants/ml were recovered from the depurinated DNA not treated with cell-free extracts. The effect on the transformation activity of control (nondepurinated) A-62 DNA treated with ISP926 (Δ) and ISP1238 (o) cell-free extracts is also shown.


100
90
80
70
60
50
40
% Transformants
0
25
50
75
100
125
Protein conc. (μg/ml)

In the DNA degradation assays described earlier (Table 19), the ngr-374-carrying strain ISP1404 demonstrated degradation of its DNA after UV irradiation. To determine the sensitivity the of ngr-374-carrying strains to UV irradiation, UV inactivation assays were performed on the ngr-374-carrying strains ISP1238 and ISP1308. Figure 14 shows the percent survivors of these strains and their parental strains when UV irradiated at 0.6 J/M^2 sec^{-1}. ISP1238 and its parental strain, ISP926, showed nearly identical rates of inactivation. In contrast, ISP1308 was approximately 20-fold more sensitive than its parental strain, ISP1297, to UV irradiation. The differences in the responses of these strains to UV irradiation was probably due to the lysogenic nature of ISP926 and ISP1238. At the dosage of UV radiation used, some or all of the prophage in the lysogenic strains were probably induced. Nonetheless, the sensitivity of ISP1308 to UV irradiation (Fig. 14), together with the results of Table 19, indicated that the ngr-374 mutation does cause UV sensitivity. However, the relationship between UV sensitivity and the affect of this mutation on the AP endonuclease activity in S. aureus is not clear.

Figure 14. UV inactivation of ngr-374-carrying and wild-type strains of S. aureus

ISP926 (ngr$^+$; □); ISP1238 (ngr-374; ■); ISP1297 (ngr$^+$; o); ISP1308(ngr-374; ●) were exposed to 0.6 J/M^2 sec^{-1}.


100
10
1.0
0.1
0.03
% Survivors
0
5
10
15
20
Time (sec)

DISCUSSION

Mapping studies

In the past, the chromosomal mapping of unselectable genes in S. aureus has been possible only when these genes could be cotransferred with selectable genes. While this method of mapping unselectable genes was successfully employed in mapping the S. aureus alpha-toxin (Brown and Pattee, 1980) and enterotoxin A (Pattee and Glatz, 1980), it can be a laborious process since many linkages may have to be examined before cotransfer of the unselectable marker can be detected. However, with the development of protoplast fusion analysis by Stahl and Pattee (1983a) a new method of genetic mapping in S. aureus has become available. The usefulness of this method is in its ability to predict linkages between unmapped and mapped markers. Although they only examined readily selectable markers, Stahl and Pattee (1983b) suggested that protoplast fusion analysis could be useful in the mapping of unselectable markers.

In this study, such an application of protoplast fusion analysis was demonstrated. Protoplast fusion analysis in conjunction with transformation was used to genetically

dissect the Rec⁻ mutant RN981. The result of this dissection was the identification of the mutations ngr-374 and mit-376, and the prediction that they were linked to thrB106 and nov-142, repectively. These predicted linkages were proved when it was shown that ngr-374 was readily cotransformable with thrB and mit-376 was readily cotransformable with his, a gene linked to nov-142. Moreover, with relatively few transformations it was possible to map ngr-374 and to propose the gene order Ω[chr::Tn551]42 - ngr-374 - thrB106. Likewise, relatively few transformations were needed to propose the gene order purA102 - nov-142 - mit-376 - hisG15 for mit-376.

It is also noteworthy that in protoplast fusions between the Rec⁻ mutants and a Rec⁺ strain, the Mod⁻ phenotype of the mutants did not seem to interfere with the recovery of recombinants even though the Rec⁺ strain was restriction-proficient. This observation suggested that DNA restriction-modification is not a factor in protoplast fusion.

mit-376

When mit-376 and hisG are cotransformed into Rec⁺ S. aureus, mit-376 causes recombinantion in the transformants to be reduced by approximately 60 to 90%; the prophage content of

the recipient influences the severity of this reduction. In addition, these transformants showed an increased sensitivity to DNA alkylating agents and MitC, and degraded their DNA after UV irradiation, a phenomenon commonly referred to as reckless DNA repair (Goering, 1979); however, the mit-376 mutation did not effect DNA modification in these transformants. These phenotypes, with the exception of DNA modification, were nearly identical to those reported for RN981 (Wyman et al., 1974; Goering, 1979). Furthermore, the recovery of Rec$^-$ transformatants from RN1441, a Rec$^+$ revertant of RN981, after the introduction of mit-376 confirmed that mit-376 was critical for the Rec$^-$ phenotype in RN981. Wyman et al. (1974) reported that recombination in RN981 was approximately 10^{-4} less efficient than in Rec$^+$ strains (i.e., 0.01%). However, most of the mit-376-carrying transformants still exhibited 10% to 40% of the recombination activity found in their Rec$^+$ parents. This relatively high level of recombination in the mit-376-carrying transformants suggested that RN981 may have multiple mutations which together are responsible for the Rec$^-$ phenotype. Based on protoplast fusion data, RN981 is known to have at least one, and perhaps two other, mutations in addition to mit-376. One of these, ngr-374, does not effect recombination. The other is a

possible UV-sensitive allele, thought to be near tmn-3106, that may reduce recombination in the mit-376-carrying strains to the level reported for RN981; however, this allele has yet to be isolated or characterized.

The possibility that RN981 carries multiple mutations that collectively are responsible for the Rec$^-$ phenotype is not unreasonable. In E. coli there are two distinct recombination pathways, RecF and RecBC (Clark, 1971). Mutations in either recB or recC reduces recombination in E. coli by approximately 90% with a concomitant appearance of MitC and UV sensitivities (Clark, 1971). The residual recombination associated with these mutants has been attributed to the RecF pathway since recombination in RecBC$^-$ RecF$^-$ mutants is reduced to the level exhibited by RecA$^-$ mutants (Horii and Clark, 1973). However, while mutations in the RecF pathway cause UV sensitivity, recombination does not decrease signficantly (Horii and Clark, 1973; Clark, 1980). Since many of the phenotypes exhibited by the mit-376-carrying strains resemble those of RecBC$^-$ E. coli, it is possible that S. aureus also has two recombination pathways: a pathway equivalent to RecBC which is impaired by mit-376, and a pathway equivalent to RecF which is impaired by the postulated UV sensitive allele near tmn-3106 in RN981.

The mit-376 mutation is also very similar to the

single-strand binding protein mutations (ssb⁻) of E. coli. Recently, the importance of the ssb protein in recombination and DNA repair of E. coli has become more apparent. ssb mutations causing decreased cell viability and recombination (Glassberg et al., 1979), increased UV sensitivity, inability to induce prophage (Johnson, 1984), and the appearance of an activity resembling reckless DNA repair (Lieberman and Witkin, 1981; Lieberman and Witkin, 1983). These phenotypes compared favorably with those of mit-376-carrying stains and suggested the possibility that mit-376 may be a mutation in S. aureus equivalent to the E. coli ssb gene. If this is the case, then RN981 would still be required to have another mutation which together with mit-376 is responsible for the Rec⁻ phenotype of RN981. This hypothesis is valid whether or not S. aureus has two recombination pathways, as suggested previously.

An entirely different possibility that is also consistent with the data is that a single mutation, mit-376, is responsible for the Rec⁻ phenotype of RN981, and that the observed discrepancy in recombination between the mit-376-carrying strains and RN981 is due to complementation of mit-376 by prophages present in the former strains. An example of a bacteriophage complementing rec mutations is provided by coliphage ϕ80. Prophage ϕ80 will complement

either recB or recC mutations in E. coli K12 by a mechanism similar to the RecE pathway encoded by the cryptic coliphage Rac in E. coli K12 (Kaiser and Murray, 1979; Reyes, 1982) which also complements recB and recC mutations. However, both of these bacteriophages require the recA protein to complement either recB or recC mutations, and in the case of the RecE pathway, an additional mutation in the sbcA gene is also required (Kaiser and Murray, 1979; Willis et al., 1984). Mechanistically these pathways appear to be analogous to the RecF pathway (Willis et al., 1984).

Given these examples of prophage-mediated complementation of rec mutations, it is conceivable that prophages in S. aureus possess similar abilities to complement, or at least to attenuate, the effect of mit-376 on recombination. However, when mit-376 was introduced into a ϕ12 lysogen (ISP1407) there was no significant difference in recombination when compared to the nonlysogens. Furthermore, a ϕ11 lysogen of RN981 has been described without mention of an alteration in the Rec$^-$ phenotype (Wyman et al., 1974). Although the ϕ11 and ϕ12 single lysogens showed no evidence of complementation, the three-fold higher recombination in ISP1314, the multiply-lysogenic mit-376-carrying strain, over mit-376-carrying nonlysogens strongly suggested that either a

combination of prophages or perhaps a cryptic prophage present in ISP1314 may be required for complementation of the mit-376 mutation. However, if the mit-376 mutation is indeed solely responsible for the Rec⁻ phenotype in RN981, then all of the nonlysogenic mit-376-carrying transformants must carry a prophage capable of complementing mit-376 in order to account for the remaining recombination in these mutants. To be consistent with the data, RN981 must then either lack this prophage or have a mutation which inhibits the ability of the prophage to complement mit-376. It is more likely that S. aureus prophages are similar to the coliphages in that they can only complement non-recA mutations, and mit-376 is not a recA mutant.

At least two possiblities exist to explain nature of the mit-376 mutation. It appears that mit-376 may be only one of the two or more mutations required for the Rec⁻ phenotype of RN981. Based on the genetics of recombination in E. coli, mit-376 could be a mutation affecting either the major recombination pathway of S. aureus (equivalent to the E. coli RecBC pathway) or the S. aureus equivalent of the E. coli ssb gene. In either case, such mutants would exhibit the phenotypes described, including the observed reduction in recombination. Alternatively, the Rec⁻ phenotype of RN981 may

indeed be due to a single mutation, mit-376, and the residual recombination observed in the mutants was caused by prophage complementation. While both of these possibilities are supported by the data, the former seems more likely since complementation of recA or recA-like mutations by a prophage has never been reported.

In conclusion, the determination of the chromosomal position of mit-376 and the effect of this mutation on recombination will serve as a foundation for further studies of recombination in S. aureus. While it is apparent that in the majority of the recipients examined, the introduction of the mit-376 does not result in Rec$^-$ transformants, this mutation can convert RN1441 to Rec$^-$. Therefore, even though it is not possible to introduce a Rec$^-$ phenotype into the genetic background of choice, it is should be possible conduct genetic manipulations with RN1441 and related strains where introduction of a Rec$^-$ phenotype is necessary.

ngr-374

The second mutation characterized in this study was ngr-374. Strains carrying this mutation were sensitive to DNA- alkylating agents, nitrous acid, and UV irradiation. However, this allele did not appear to influence cell

viability or genetic recombination.

Based on current models for DNA repair, the DNA lesions produced by the above agents are mainly repaired by different forms of excision repair which probably share a common DNA polymerase and DNA ligase (Lindahl, 1979; Friedberg et al., 1981). Based on phenotype, the ngr-374-carrying strains were believed to be deficient in AP endonuclease. Bioassays which measured AP endonuclease activity in cell-free extracts showed that the ngr-374-carrying strain had low levels of AP endonuclease activity compared to the wild-type strain; however, by increasing the amount of cell-free extract from the ngr-374-carrying strain in the reaction mixture it was possible to detect AP endonuclease activity. This detection of AP endonuclease activity was similar to the results reported for xthA mutants in E. coli which lack the AP endonuclease Exo III (Ljundquist et al., 1976). Using a similar assay, Ljundquist et al., (1976) reported the detection of AP endonuclease activity as increasing amounts of cell-free extracts from the xthA mutant of E. coli were assayed. This detection of AP endonuclease activity was attributed to the presence of other endonucleases having limited AP endonuclease activity in the cell-free extracts (Ljunquist et al., 1976; Warner et al., 1980; McMillan et al.,

1981). A similar conclusion was drawn from the data for ngr-374. Furthermore, ngr-374 did not significantly alter cell viability or their ability to reactivate MMS-treated bacteriophage 80α, supporting the suggestion that other cellular nucleases with limited AP endonuclease activity compensated for the ngr-374-induced deficiency in AP endonuclease activity in S. aureus (Ljundquist et al., 1976; Karren et al., 1980; Warner et al., 1980; Stuy and Bagci, 1983).

Upon first examination, the conclusion that ngr-374 caused a deficiency in the S. aureus AP endonuclease was inconsistent with the UV sensitivity exhibited by these mutants. The repair of DNA lesions generated by UV irradiation is usually thought to occur via the nucleotide excision repair mechanism, which does not form AP sites and does not require an AP endonuclease (Friedberg et al., 1981; Lindahl, 1982). Therefore, a mutation affecting AP endonuclease activity would not be expected to cause sensitivity to UV irradiation. However, a few examples exist where base excision occurs in the repair of UV-generated DNA lesions. The enzymes responsible for this type of repair have been found in coliphage T4 and Micrococcus luteus (Gordon and Haseltine, 1980; Lindahl, 1982). They are single peptides which possess

both a thymine dimer specific DNA-N-glycosylase and AP endonucleolytic activity (Tomilin et al., 1976; Gordon and Haseltine, 1980; McMillan et al.,1981). More recently, evidence has been presented that in E. coli and human lymphocytes repair of a minor product of UV irradiation, 5,6-hydrated thymine (thymine glycol), occurs by an AP endonuclease-dependent base excision mechanism (Demple and Linn, 1980; Lindahl, 1982; Brent, 1984). These reports of base excision repair of UV-generated lesions suggest that it is possible for S. aureus to have an AP endonuclease-dependent base excision repair mechanism specific for UV-generated DNA damage which is separate from repair of UV damaged DNA by nucleotide excision. This suggestion was supported by the response of ngr-374-carrying strains to NQO and the studies of Wyman et al. (1974) on the Rec$^+$ revertants of RN981.

NQO has been shown to mimic UV irradiation so closely that mutants of E. coli and B. subtilis impaired in UV excision repair (i.e., nucleotide excision) were sensitive to this compound (Kondo et al., 1970; Ikenaga et al. 1981; Dai et al., 1983; Friedman and Yasbin, 1983). In S. aureus, the uvs mutations are believed to impair UV excision repair based on their sensitivity to UV irradiation and inability to reactivate UV irradiated bacteriophage (Goering and Pattee,

1971); like their counterparts in E. coli and B. subtilis, these uvs mutations also cause sensitivity to NQO (unpublished results). In contrast, ngr-374-carrying strains were not sensitive to NQO, which suggests that this mutation was not in one of the uvs genes. This conclusion is further supported by Wyman et al. (1974), who reported that RN1440 and RN1441, strains shown in this study to carry ngr-374, were UV sensitive, but were unaffected in their ability to reactive UV-irradiated bacteriophage. Based on the observations of Wyman et al. (1974) and the response of ngr-374-carrying mutants to NQO, it appears that ngr-374 does not impair the same UV repair system as the uvs mutations, but probably interferes with a unique AP-endonuclease dependent repair process.

In summary, it has been shown that S. aureus has an AP endonuclease and that ngr-374, isolated from the Rec$^-$ mutant RN981, reduces the AP endonuclease activity in S. aureus. Judging from the sensitivity of these strains to DNA alkylating agents, this mutation probably impairs the major AP endonuclease in S. aureus. While this mutation causes sensitivity to UV irradiation, it failed to elicit any of the phenotypes characteristic of mutants of UV excision repair (Goering and Pattee, 1971), suggesting that S. aureus may have

multiple repair mechanisms for UV-generated lesions, including one which requires the AP endonuclease affected by ngr-374. Finally, it is not known whether ngr-374 was a mutation in the structural gene for the AP endonuclease or in a gene which regulates the expression of this enzyme. Resolving this issue will require a more extensive examination of the genetics of this type of DNA repair in S. aureus.

SUMMARY

Two mutations have been identified and isolated from the Rec⁻ mutant RN981. The mit-376 mutation was identified by protoplast fusion analysis and was shown by genetic transformations to be in the linkage group purA102 - nov-142 - mit-376 - hisG15 (Fig. 9, segement 10). When transformed into Rec⁺ recipients, mit-376 caused an increase in the sensitivity to MitC, DNA alkylating agents, and UV irradiation. Associated with UV sensitivity was the appearence of reckless DNA repair. In addition to these phenotypes, mit-376 was shown to convert the Rec⁺ revertant of RN981 to Rec⁻. In addition, mit-376 reduced genetic recombination in strains that were either non-lysogenic or that carried only one prophage by approximately 90%, but only by 60 to 70% in a multiple lysogen. While these reductions were significant, the levels of recombination in the mit-376-carrying strains were more than 1000-fold greater than that reported for RN981 (Wyman et al., 1974). Based on these results, it was concluded that mit-376 was only partly responsible for the Rec⁻ phenotype of RN981 and might be a mutation in the S. aureus equivalent to the E. coli recB, recC, or ssb genes, rather than the E. coli recA gene as suggested by Wyman et al. (1974).

The second mutation described in this study was ngr-374. Like mit-376, ngr-374 was identified in RN981 by protoplast fusion analysis and shown by genetic transformation to be in the linkage group Ω[chr::Tn551]42 - ngr-374 - thrB106 (Fig. 9, segment 16). This mutation caused sensitivity to DNA alkylating agents, nitrous acid, and UV irradiation, but had no effect on genetic recombination. Bioassys for AP endonuclease activity showed that ngr-374 caused a reduction in the activity of the AP endonuclease; however, it was apparent that this deficiency was compensated for by other S. aureus nucleases. Furthermore, the UV sensitivity suggested that S. aureus has two repair pathways for UV-generated DNA damage: the UV excision pathway described by Goering and Pattee (1971) and a unique pathway which is AP endonuclease-dependent.

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